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RATIONS FOR BOBWHITE QUAIL*

W. J. Stadelman,† E. W. Callenbach, R. R. Murphy, and R. V. Boucher‡

A Report on Some Nutritional Experiments

PRACTICAL skill, sometimes designated art, is and probably will continue to be *the* factor of paramount importance in attempts of man to propagate living things. Frequently little or no scientific information is needed for small scale production. However, when propagation is attempted as a commercial venture so many problems are encountered that the substitution of facts for opinionated information becomes a practical necessity.

In recent years large scale propagation of bobwhite quail has been undertaken on private and public game farms. This has resulted in (1) an accentuation of old difficulties, and (2) the appearance and recognition of a host of new troubles associated with the unnatural conditions accompanying large concentrations of populations in restricted quarters. Specific environmental problems encountered include provision of proper and adequate nutrition, shelter, and sanitation, with adaptation of rearing procedures to the disposition and instincts of the species. This paper is concerned with nutrition.

Very little factual information on the dietary requirements of artificially reared bobwhite quail is available. LeCompte (1931), reporting early work in Maryland, itemized a feeding schedule which included: First feeding at 24 hours; clabbered milk throughout rearing period; closed formula commercial starting and growing mashes (whether chicken, turkey, pheasant, or quail not stated); green feed; sand and grit; and a commercial grain mixture (closed formula). A year later Handley (1932) reported that W. B. Coleman had successfully reared quail by mash feeding without either clabber or boiled egg. No mention was made of green feed or other dietary supplements. At the same time MacNamara (1932) reported success in rearing quail on a mash ration without clabber or boiled egg. He did not indicate whether supplementary feeding of green feed or other materials was practiced. MacNamara's starter mashes had calculated analyses of 17.30 and 19.15 per cent digestible crude protein. Growing mashes, used in conjunction with the starters, were calculated to contain 19.20 and 20.42 per cent digestible crude protein. It should be emphasized that these protein levels are not for crude protein but for *digestible* crude protein, the digestibility figures being based on investigations involving four-footed domestic animals and not game birds or more nearly related domesticated poultry. A year later MacNamara (1933) pointed out that some quail breeders were using mashes "having a protein content from 20 to almost 30 per cent."

Sometime later Petty (1934) reported excellent results from the use of several closed formula commercial chick, turkey, and quail mashes with and without supplements of lettuce or fermented alfalfa meal. Whether other supplementary feeds or special feeding procedures were involved in Petty's work is not clear.

In none of the publications referred to has any specific information relative to formulae been given. Likewise, with the exception of LeCompte's work, it is not known how many or what kinds of nutritional supplementation and modification or changes in the details of rearing management during the rearing season were involved. These facts are important for, as indicated later under Experimental Procedure, quail raisers are apparently accustomed to all sorts of additions and variations in diets and mechanics of management, any or all of which are more or less disregarded in verbal or written discussions but which may vitally influence results achieved and a correct interpretation of differences between groups.

The writers have found only three publications reporting definite experiments in bobwhite quail nutrition. Norris (1935) is credited with concluding that "27 per cent of protein was necessary for the most rapid growth of the chicks of this species. However, the difference between the results obtained at the 27 per cent level of protein and the 24 per cent level of protein was not great." Seven years later Nestler and associates (1942) published composite results of four experiments on protein requirements. Their report stated that a 28 per cent level of protein intake gave best general results during a rearing period of 10 weeks. An interesting point emphasized was that 45 per cent mortality during the rearing period is considered normal by bobwhite breeders. In later work, Nestler et al. (1944) were able with small groups of chicks, to reduce six-week mortality to 18 and 19 per cent on three diets and 10 weeks mortality to 22 and 20 per cent on two of the same diets.

Experimental Procedure

The two experiments described herein were conducted at the Pennsylvania Game Commission's Fisher State Game Farm, Schwenksville, Pa. Since the farm is located 180 miles from the Pennsylvania State College, experimental observations were limited to bi-weekly visits by the writers. A careful record of chick behavior, management procedures and any occurrences considered to be unusual or of experimental interest was made for each experimental group by the caretaker or game farm superintendent. Growth data, regular periodic observations of environmental conditions, and feed consumption data were obtained during the scheduled visits of the writers.

Figure 1. Quail brooder house with attached wire-floored outside runs.

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The assistance of V. T. Warfel, Superintendent of the Fisher State Game Farm, Schwenksville, and his assistants in the prosecution of these experiments is gratefully acknowledged.

As intimated in the review of literature, bobwhite quail management usually includes many procedures not always discussed completely in reports of rearing results. Consequently, certain practices, taken for granted in the standard quail rearing procedure at the state game farm, but inimical to most accurate experimentation, were placed in effect without previous knowledge or formal approval of the writers. Since, with a few exceptions as noted, the unscheduled procedures were constant for all pens, the comparative data assembled are completely valid for accurate interpretation. However, from the standpoint of practical application of results, the best feeding and management findings must remain too complicated and too cumbersome for greatest satisfaction. It is hoped that further experiments may result in the solution of certain critical problems and greatly simplify rearing technique.

Both experiments were conducted in the long quail brooder house illustrated in Figure 1. Each wing, on either side of a central feed room, is 56 feet long, 20 feet deep and 8 feet high at the walls. Each inside brooder pen is 3′ x 7′ x 12″, the area occupied by the electric heating unit being 3′ x 3′ x 12″. Each outside run, as shown in the illustration, is 3′ x 8′ x 12″.

Inside pen floors are 3-mesh-per-inch hardware cloth supported over metal droppings pans, kept bedded down with shavings. The outside runs are floored with 2-mesh-per-inch hardware cloth supported over droppings pans not bedded but washed clean at frequent intervals. Replicate electric heating units and the same kinds, sizes, and numbers of feeders, water fountains, etc., were used for all groups of chicks. A representative complement of equipment for a group of chicks is shown in Figures 2 and 3.

Experiment One. Twenty-two groups of 100 12-hour-old bobwhite quail chicks each were placed in brooding quarters as described, on the afternoon of May 28, 1942. For purposes of convenience, duplicate groups located on opposite sides of the central brooder house alleyway were designated a and b. Mash formulae are given in Table 1. Identification of groups for number, mash mixture fed, and chemical composition, by analysis, of the mash mixtures is shown in Table 2. The standard feeding procedure was as follows:

Maxco, a 20 per cent protein commercial mixture of cooked wheat flour and 68 per cent protein meat scraps, was mixed in the ratio of 8:1 with cod liver oil having a guaranteed potency of 200 A.O.A.C. chick units of Vitamin D and 1500 U.S.P. units of Vitamin A per gram of oil. The Maxco and oil were mixed thoroughly, scalded with water, and dried to a crumbly mass by the addition of a sufficient quantity of the mash mixture fed to the particular group for which the Maxco was intended. Decreasing quantities of Maxco and increasing quantities of mash were added daily until at two weeks the Maxco was discontinued entirely, the dry mash alone being available thereafter. The Maxco mixture was fed 2 to 4 times daily, the number of feedings per day being decreased until stopped completely when the quail were two weeks old. The Maxco was used chiefly because its texture and appearance attracts young chicks and stimulates feed consumption. It also acts as a vehicle for the Vitamin D oil until mash consumption will take care of the Vitamin D requirement of the chicks.

The appropriate group mash mixture was available constantly in small flat pans. On and after the second day ground lettuce, and later leaves of lettuce, was fed. On and after June 5, when the quail were 8 days old, charcoal was sprinkled on the mash whenever feed pans were filled. The chicks were flushed with epsom salts on June 10, 16, and 29 because several groups showed symptoms of constipation. All groups were flushed to maintain uniformity of management procedure except that on June 10, through an oversight, groups 11a and 11b were not treated. The epsom salts solution used was made up of 1 tablespoonful of the salts to each 1 gallon of water.

Except as noted later in the discussion of results, feeding and other management procedures were constant for all pens. Thus, while the general management system was rather complex, the only major environmental variable was the difference in mash mixture provided.

Experiment Two. Twenty-two hundred day-old chicks divided into groups as in Experiment One were placed in the same brooding quarters on July 16, 1942. The mash mixtures fed, in part duplicates of several used in Experiment One, were based largely upon observations made during the earlier test and the results of previous work with Ring-necked pheasants. Table 4 gives the mash formulae used and Table 5 shows their chemical composition. As indicated in this table (groups 20 and 21), an attempt was made to eliminate the Maxco moist mash feeding during the early life of the chicks. Except as indicated otherwise, all groups were fed in a manner similar to those in the first experiment. Epsom salts was fed on July 31.

Results and Discussion

An indicated under Experimental Procedure, all treatments were run in duplicate. Since careful examination of tabulated results showed few important differences between the duplicate groups, the data as presented here are for combined groups considered as single units. Measurements used to evaluate the rations fed were: (1) growth, (2) feed usage (3) mortality and (4) quality of birds reared.

Experiment One. Results for this experiment are summarized in Table 3. On the basis of weights at 6 weeks, groups, 7, 6, 11, and 10 in the order named were distinctly superior. The same groups, rearranged with 11 and 10 ahead of 6 and 7, experienced, in order, the lowest mortality and therefore best livability. In feed usage, groups 6 and 7 required virtually the same and the largest amounts. Groups 10 and 11 used far less feed than might be expected in comparison with other groups. This is easily explained.

The mashes fed to groups 1 to 8 inclusive were similar in physical structure to usual poultry mashes. Groups 10 and 11 were fed the "kernel" mixtures in which the ingredients were processed to form minute granules. Since shallow open pans and hoppers were used for feeding the mash mixtures (Figure 2), the quail could scratch or dust in them. It was observed that feed wastage, which could not be measured with any degree of accuracy, was quite heavy in the ordinary mash-fed pens and much less in the "kernel"-fed pens. This is a repetition of previous similar experience with pheasants. Because of the feed wastage no indices of feed utilization efficiency were calculated, and the term feed usage is used instead of the more familiar feed consumption. It should be possible, although admittedly difficult because of the small size and great activity of quail, to devise feeders which will greatly reduce feed loss and thus make possible more accurate determination of the nutritive value of mash mixtures. In general, and with the exception of "Kernel"-mixture consumption as noted, the data in Table 3 indicate clearly a close relationship between growth, feed consumption and mortality. They also show conclusively that mash mixtures 6, 7, 10, and 11 fed to groups similarly numbered were the best mixtures used, with little to choose among them. It may be mentioned in passing that both open formula mash mixtures were sufficiently lower in cost than the "kernels" to more than compensate for the feed wastage, and therefore greater feed usage, described.

Figure 2. Feeding and watering equipment used for each group of 100 quail chicks.

Experiment Two. The five most promising mash mixtures used in Experiment One were fed again in Experiment Two. In addition three mixtures found previously to have been excellent for Ring-necked pheasants (Stadelman and associates, 1944), and one new granular type mash were added.

Results for Experiment Two are summarized in Table 6. Although the groups of birds as a whole were more uniform in this test, there was, as in Experiment One, a distinct segregation on the basis of superiority as determined by the measurements made. Bird quality as acertained by size, apparent health, plumage condition, freedom from injury, etc., was an added measurement of differences between the rations used in Experiment Two.

Mash mixtures 6, 7, 10, and 11 again gave reasonably good results for late-hatched chicks. However, the results were not equal to those secured with pheasant mixtures 12, 13, and 14 fed to groups 15, 16, and 17. While differences in growth, feed usage, and number and quality of birds reared were negigible among the latter three groups, the mortality of group 17 was distinctly the lowest. Mixture 14, fed to this group, had, when used for pheasant chicks hatched in May of the same year, given an exceptionally low mortality, 1.6 per cent to 6 weeks.

It may be observed, Tables 4 and 5, that this superior mixture was simple and modern. It contained 40 per cent of soybean oil meal, the protein level was approximately 29 per cent, and the calcium and phosphorus levels were 1.31 and .84 per cent, respectively, this giving a Ca:P ratio of 1.56:1.

Groups 20 and 21, fed mash mixtures 6 and 7 without the moist Maxco supplement, were inferior to groups 13 and 14 fed the same mashes but given the supplement in addition. However, the only important difference was in early mortality. This was apparently due to the chicks getting mash in their eyes, the eyes then becoming pasted shut, a condition which stimulated eye-picking by the more vigorous birds. This condition lends weight to the suspicion that the Maxco feeding may be of more value mechanically than nutritionally.

Mixture 3, fed to group 19, again gave inferior results. Granular mixture 15, fed to group 19 gave moderately good growth and livability and showed a feed usage comparable to that of the "kernel" pens.

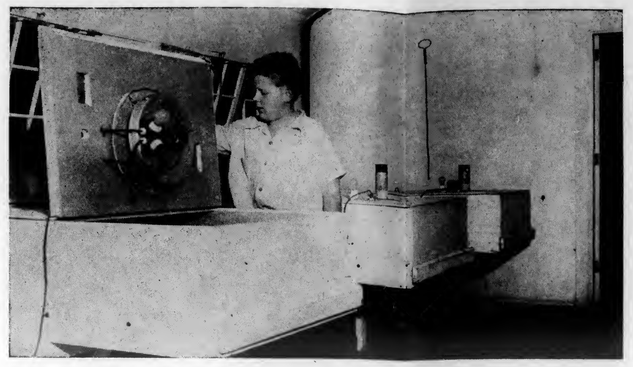

Figure 3. Electric brooder heating assembly used for each group of 100 quail chicks.

TABLE 1—COMPOSITION OF MASH MIXTURES USED IN EXPERIMENT ONE (In Pounds)

(To each was added: salt, 0.5 lb.; cod liver oil (400 A.O.A.C.), 0.25 lb.; and $MnSO_4$, 0.025 lb. Each ration totalled 100.275 pounds.)

Group	1	2	3	4	5	6	7	8	9	10	11
Ground yellow corn	20.0	20.0	15.0	30.0	33.5	11.2	17.5	26.0	25.0		
Wheat bran	15.0	5.0	15.0	15.0	15.0	15.0	10.0	10.0	10.0		
Wheat flour middlings	12.5	...	15.0	15.0	15.0	12.5	10.0	10.0	10.0		
Ground oats	10.0	15.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0		
Alfalfa leaf meal	5.0	10.0	5.0	7.5	7.5	5.0	5.0	5.0	7.5		
Dry skim milk	12.5	10.0	9.5	2.5	7.5	12.5	8.0	10.0	5.0		
Soybean oil meal	...	6.5	15.0	10.0	...	19.5	12.0	5.0	12.0		
White fish meal	11.0	10.0	10.0	2.5	5.0	2.75	...	5.0	10.0		
Menhaden fish meal	...	...	...	...	...	...	15.0	...	...		
Meat scraps (50% protein)	...	...	...	2.5	5.0	11.05	...	11.5	5.0		
Meat scraps (55% protein)	...	5.0	5.0	...	...	...	12.0	...	...		
Meat scraps (65% protein)	13.5	...	...	...	...	...	...	...	...		
Ground limestone	...	...	...	1.0	1.0	...	...	2.0	2.0		
Oyster shell flour	...	3.0	...	...	...	...	...	...	...		
Steamed bone meal	...	...	...	1.0	...	...	...	...	...		
Ground wheat	...	15.0	...	...	...	...	...	...	...		
Corn gluten meal	...	...	...	...	...	...	...	5.0	...		
Riboflavin concentrate	...	...	...	2.5	...	...	...	...	...		
Dried brewers' yeast	...	...	...	...	...	...	...	...	3.0		
Commercial "Kernel" mixture Guaranteed analysis—30% protein											
Commercial "Kernel" mixture Guaranteed analysis—26% protein											

Discussion and Practical Applications

It would be a waste of time to analyze statistically the results secured in the two experiments reported. Valid, ordered information about bobwhite quail chicks is still so limited and management procedures with sizeable groups of chicks are so complex and varied that results are influenced by too many environmental factors to permit accurate analysis. However, sufficiently large populations were used in these tests (100 chicks per experimental group; 200 per treatment group as reported in the tables) to give ample evidence by means of which the mash mixtures studied can be easily classified as poor, fair, and good.

It would be manifestly unfair to compare the rations used in Experiment One with different rations used in Experiment Two. It would also be objectionable and faulty to attempt a severe comparison between results from the same mash mixtures used in both tests. It is justifiable to examine closely comparative results secured with different groups of chicks hatched at different periods and brooded in different pens but fed mash mixtures based on the same formula. Such results may also be compared with similar results obtained with other mixtures fed in conjunction with these basal mixtures fed in each experiment.

In line with this reasoning mash mixtures 6, 7, 10, and 11, fed to groups 6 and 13, 7 and 14, 10 and 12, and 11 and 22 respectively, serve as bases for comparison of the fifteen experimental mixtures studied. Because of the difficulty experienced in feed usage, comparisons discussed here are limited primarily to growth and mortality supplemented by bird quality as measured in Experiment Two.

In Experiment One, the groups fed rations 6, 7, 10, and 11 were so much better than all other groups in both weight and livability that there is no doubt of their superiority. As a matter of fact, not a single 100-chick group on the other rations averaged in weight within 4 grams of the smallest average in any similar group fed these four best rations. The smallest mortality in any 100-chick group fed the other rations was 25 per cent, the greatest in any of the 100-chick best ration groups being 14 per cent.

In Experiment Two, in which the six poorest rations of Experiment One were replaced, mixtures 6, 7, 10, and 11 again gave reasonably good results. However, they did not compare favorably with mixtures 13, 14, and 15 in either growth, mortality or number of

Figure 4. Brooder pens; the droppings pan, bedded with shavings, of one pen is partly withdrawn from under the wire floor.

TABLE 2—CHEMICAL ANALYSES OF MASH MIXTURES USED IN EXPERIMENT ONE (In Percentages)

Group	Maxco	1	2	3	4	5	6	7	8	9	10	11
Moisture	8.06	8.53	7.80	8.23	8.56	8.90	9.11	8.07	7.98	8.99	9.01	8.14
Ash	2.68	13.15	13.89	11.89	8.75	9.90	9.80	8.68	11.47	13.91	7.98	9.95
Ether Extract	3.36	5.68	4.36	4.76	4.64	4.94	4.30	5.58	4.84	4.65	6.23	7.53
Protein	23.69	23.69	19.19	23.38	18.69	17.19	28.00	31.94	23.13	21.25	24.88	27.56
Crude fiber	1.46	5.60	5.84	5.98	6.87	6.04	5.98	5.07	5.46	5.76	4.93	4.57
Calcium	.29	3.56	4.07	3.17	1.77	2.34	2.08	1.81	3.19	3.86	1.85	2.27
Phosphorus	.57	1.22	1.60	1.35	1.08	1.24	1.21	1.25	1.54	1.28	0.95	1.20
Managnese	.0034	.0103	.0088	.0100	.0110	.0114	.0127	.0133	.0116	.0117	.0103	.0091
Ca:P ratio	.51:1	2.92:1	2.54:1	2.35:1	1.64:1	1.89:1	1.72:1	1.45:1	2.07:1	3.01:1	1.95:1	1.89:1

good birds reared. They were again distinctly better than mixture 3 and mixtures 6 and 7 fed without scalded Maxco, but were no better than mixture 15. Group 13, fed mixture 6, was more deficient on all counts than would be expected. This was due to definitely poorer growth and higher mortality in pen 13b. No reason is known for this condition.

Summary

Four thousand four hundred day-old quail chicks were used to evaluate 15 mash mixtures. On the basis of all available evidence, it may be concluded that: 1. Mash mixture 14, fed to group 17, was the best feed offered as measured by weight, livability and number or per cent of good birds reared to 6 weeks of age.*

2. Mixtures 12 and 13, fed to groups 15 and 16, were about as good as mixture 14 in growth, feed usage, and number of good birds reared, but somewhat poorer in mortality results.

3. Mixtures 7 and 11 were satisfactory quail feeds but did not measure up to mixtures 14, 12, and 13. Mixtures 10, 6, and 15 were also satisfactory.

4. Mixtures 1, 2, 3, 4, 5, 8, and 9 were definitely unsuited for quail.

With the exception of 5424 chicks in two groups in which the mortality, definitely due to experimental management procedure and inexperienced brooder attendants, was 27.4 and 25.44% respectively, the remaining 15,759 chicks started completed the six-week brooding period with a mortality of only 1788 birds or 11.34%. The results obtained from late hatched birds merits special note. The last four hatches of the season totalled 8,190 chicks hatched July 6th to July 27th. Of these 3,898 were brooded in the brooder house shown in Figure 1,—3,629 in another big brooder house, and 663 in colony houses. These chicks completed the six-week brooding period with a total mortality of 854 birds or 10.43%.

(Footnote prepared by V. T. Warfel, Superintendent of Fisher State Game Farm.)

* Since these experiments were completed the Pennsylvania Game Commission has reared many thousands of quail on ration 14. In 1944, 17,383 quail were raised with a total brooder mortality of only 15.31 per cent to six weeks of age. This was not only the lowest qail mortality for any brooding season on the Fisher State Game Farm, but the quality of the birds at six weeks, based on vigor, uniformity, and good feathering, was the best.

TABLE 3—BODY WEIGHTS, FEED USAGE AND MORTALITY, BY TREATMENTS; EXPERIMENT ONE

Group No.	Mash No.	Average weight in grams at Day-old	Average weight in grams at 6 weeks	Feed usage in pounds per 100 chicks to six weeks	Mortality in per cent to six weeks
1	1	6.52	65	53.5	51
2	2	6.52	52	45.7	59
3	3	6.10	76	58.6	33
4	4	6.24	61	48.8	29
5	5	6.10	49	37.2	53
6	6	6.24	89	63.6	12
7	7	6.38	96	63.1	12
8	8	6.38	70	53.0	46
9	9	6.31	61	49.5	29
10	10	6.24	86	48.2	11
11	11	6.24	87	46.7	7

TABLE 4—COMPOSITION OF MASH MIXTURES USED IN EXPERIMENT TWO (In Pounds)
(To each was added: salt, 0.5 lb.; cod liver oil (400 A.O.A.C.), 0.25 lb.; and $MnSO_4$, 0.025 lb. Each ration totalled 100.275 pounds)

Group	12	13	14	15	16	17	18	19	20*	21*	22
Mash Mixture	10	6	7	12	13	14	15	3	6	7	11
Ground yellow corn	11.2	17.5	12.5	16.5	9.5	...	15.0	11.2	17.5	...	
Yellow cornmeal (bolted)	...	...	...	...	...	29.5	...	...	...	...	
Wheat bran	15.0	10.0	10.0	10.0	10.0	15.0	15.0	15.0	10.0	10.0	
Wheat flour middlings	12.5	10.0	10.0	10.0	10.0	...	15.0	12.5	10.0	10.0	
Ground oats	10.0	10.0	10.0	10.0	10.0	...	10.0	10.0	10.0	10.0	
Alfalfa leaf meal	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	
Dry skim milk	12.5	8.0	...	...	...	10.0	9.5	12.5	8.0	...	
Dried whey	...	...	10.0	5.0	5.0	...	...	...	...	...	
Soybean oil meal	19.5	12.0	30.0	25.0	40.0	20.0	15.0	19.5	12.0	...	
Corn gluten meal	...	...	...	...	...	5.0	...	...	...	...	
White fish meal	2.75	...	...	...	...	5.0	10.0	2.75	...	...	
Menhaden fish meal	...	15.0	4.0	3.0	2.0	...	...	...	15.0	...	
Meat scraps (50% protein)	11.05	...	8.0	12.0	3.0	10.0	...	11.05	...	...	
Meat scraps (55% protein)	...	12.0	...	...	...	...	5.0	...	12.0	...	
Ground limestone	...	...	...	...	1.0	...	...	...	...	...	
Steamed bone meal	...	...	...	...	1.0	...	...	...	...	...	
Dried brewers' yeast	...	...	...	3.0	3.0	...	...	...	...	...	

Commercial "Kernel" mixture Guaranteed analysis—28% protein

Commercial "Kernel" mixture Guaranteed analysis—30% protein

* No scalded Maxco feeding was practiced with groups 20 and 21.

TABLE 5—CHEMICAL ANALYSES OF MASH MIXTURES USED IN EXPERIMENT TWO (In Percentages)

Group	12	13	14	15	16	17	18	19	20*	21*	22
Mash Mixture	10	6	7	12	13	14	15	3	6	7	11
Moisture	10.02	10.06	10.06	10.15	10.42	10.35	9.74	9.56	9.86	9.80	9.27
Ash	7.90	9.51	8.62	7.54	8.24	7.40	9.66	12.06	9.34	8.93	9.56
Ether Extract	6.31	3.73	5.68	4.16	4.33	2.91	3.42	4.30	4.68	6.05	6.49
Protein	24.88	25.44	29.94	27.44	27.31	28.94	25.31	21.56	25.81	28.13	28.69
Crude fiber	4.77	6.60	5.70	6.75	6.80	7.28	5.01	6.16	6.70	6.69	4.26
Calcium	1.79	2.16	1.96	1.22	1.72	1.31	2.58	3.25	2.09	2.00	2.31
Phosphorus	0.86	0.92	0.94	0.84	0.93	0.84	0.87	0.90	0.89	0.88	0.87
Manganese	.0113	.0102	.0108	.0100	.0124	.0110	.0084	.0126	.0102	.0126	.0108
Ca:P ratio	2.08:1	2.35:1	2.09:1	1.45:1	1.85:1	1.56:1	2.97:1	3.61:1	2.35:1	2.27:1	2.66:1

* No scalded Maxco feeding was practiced with groups 20 and 21.

TABLE 6—BODY WEIGHTS, FEED USAGE, MORTALITY AND PERCENTAGES, GOOD BIRDS REARED, BY TREATMENTS; EXPERIMENT TWO

Group No.	Mash No.	Average weight in grams at Day-old	Average weight in grams at 6 weeks	Feed usage in pounds per 100 chicks to 6 weeks	Mortality (per cent to 6 weeks)	Good birds at 6 weeks (No.)	Good birds at 6 weeks (Per cent)
12	10	6.38	78	46.4	31	131	94
13	6	6.24	72	62.5	41	93	79
14	7	6.38	84	61.8	30	136	95
15	12	6.52	88	64.7	19	162	99
16	13	6.52	91	66.0	18	162	95
17	14	6.52	91	66.3	13	168	97
18	15	6.24	79	51.7	33	115	91
19	3	6.24	63	65.4	55	51	57
20	6*	6.52	78	64.8	52	90	92
21	7*	6.24	75	63.7	44	98	88
22	11	6.24	84	51.7	25	142	97

* No scalded Maxco feeding was practiced with groups 20 and 21.

Severe winters deplete the ranks of Bobwhite.

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Some Present Day Aspects Of Livestock Nutrition

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SOME PRESENT DAY ASPECTS OF LIVESTOCK NUTRITION*

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The nutrition of yesterday involving simply the utilization of fats, carbohydrates, protein and a few mineral elements has now grown to a science with innumerable aspects, even a few of which could not be reviewed thoroughly in a short discussion. Therefore this paper is limited to a few facts concerning some recent developments in the nutrition of livestock which may be of general interest.

The field of vitamin chemistry has expanded to such an extent that almost a score of factors have been isolated and identified, all of which exhibit characteristic vitamin activity. Approximately half of these factors are now produced synthetically and are available for clinical use. In many instances, however, the usefulness of these vitamins in clinical practice is limited by a lack of knowledge not only of their mode of action, but also of the pathology attending specific deficiencies. The situation is even worse in the case of multiple deficiencies. In other instances enough progress has been made so that specific vitamin deficiencies are easily recognized and treated.

It is now recognized that vitamin A deficiency is one of the most common vitamin deficiency diseases of cattle and may occur not only in the calf, but in cattle of all ages. A common symptom of vitamin A deficiency is night blindness resulting from inability of the animal to regenerate sufficient visual purple or rhodopsin, a light sensitive pigment present in the retina, which is a compound of vitamin A and protein. Simple night blindness may be cured by the administration of vitamin A. However, if untreated, permanent blindness may ensue owing to constriction of the optic nerve. The changes involved are stenosis of the optic foramen caused in part by proliferation of cranial bone and by edema of the optic nerve resulting from increased cerebrospinal pressure. Blindness which is related to vitamin A deficiency may occur in cattle, horses, sheep, swine and dogs.

Another symptom of vitamin A deficiency in cattle is anasarca, a swelling of the legs and forequarters. The edema

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usually involves the legs, brisket and shoulders but may become generalized. Other symptoms of vitamin A deficiency are rapid respiration, stiffness or lameness, convulsions and a loss of appetite and weight.

Vitamin A is also essential for reproduction in cattle. This deficiency may result in abortion, dead, paralyzed or weak calves or, in the more advanced stages, complete impairment of reproduction which is not necessarily permanent if properly treated.

The newborn calf has very low reserves of vitamin A. If placed on a vitamin A deficient diet it will die within 30 to 60 days with scours and pneumonia. However, the ingestion of colostrum affords an opportunity for the calf to accumulate a considerable reserve of vitamin A which is deposited in the liver. The A content of the blood plasma rises five fold in 24 hours with the intake of colostrum, which is 10 to 100 times richer in vitamin A than ordinary milk. The calf should receive colostrum for two or three days, not just for 12 hours, in order recognized, of course, that vitamin A is only one of a number of to obtain the full benefit of this additional vitamin A. It is beneficial factors which the calf obtains from colostrum.

Supplementary vitamin A can be given to calves in the form of a vitamin A concentrate or an oil such as shark liver oil in daily dosages up to 5000 units. There are some indications that the newborn calf cannot use carotene, the precursor of vitamin A. A good grade of hay such as U. S. No. 2 is usually sufficient to provide the needs of mature cattle for vitamin A.

The situation with respect to the needs of the calf for vitamin D is relatively simple although rickets and bone deformities in calves are still rather commonly seen. The growing calf requires the bone forming elements calcium and phosphorus in the proper ratio, about 1.2:1, and enough vitamin D to insure their proper utilization. Usually sun cured hay supplies enough vitamin D for this purpose. Exposure of the calf to sunlight, even in winter, supplies some vitamin D. Additional vitamin D may be provided cheaply by vitamin D yeast a product which is included in some proprietary calf meals. Less digestive troubles have been reported from experiments in which vitamin D was supplied by cod liver oil; however, evidence on this point is not conclusive.

Judging by the blood level of vitamin C, it is apparent

that the tissues of the newborn calf are fairly high in this vitamin. However, the vitamin C content of the blood decreases very rapidly in the first 24 hours of the life of the calf and continues to drop more slowly thereafter for about 14 days. Since one of the functions of vitamin C appears to be as an aid to body defenses in warding off infections, it has been found that the feeding of 0.25 to 0.50 grams daily of ascorbic acid is of value in preventing peritonitis, and for calves with enlarged navels and other infections. The blood vitamin C level can be maintained by feeding ascorbic acid for only a period of a week or 10 days owing apparently to destruction of the vitamin in the developing rumen. After this time it is necessary to inject the vitamin. Normally the vitamin C blood level in the calf begins to rise within two to three weeks after birth.

It has been indicated that while a skim milk diet supplemented with vitamins A, D and C supports life in the very young calf it is claimed that the addition of niacin is necessary for the prevention of scours. Fifty to 100 mg. of niacin daily are indicated for a young calf. Niacin seems to be valuable both in the treatment and prevention of enteric disorders in the calf as well as in other animals.

Apparently there is still some confusion in differentiating between scours in calves owing to nutritional deficiency and acute scours in the newborn calf of the infectious and contagious type which usually are not followed by pneumonia. The possibility exists that the latter may possibly be predisposed in part by faulty nutrition. However, nutritional treatment of severe scours in the very young calf has been virtually ineffective in most instances and in this type of scours treatment with sulfa drugs is indicated.

Very little is known concerning interrelationships between vitamins. However, evidence on this point is being accumulated and is well illustrated by the synergistic effect of vitamin E (alpha tocopherol) and vitamin A. It has been shown with experimental rats that the utilization of vitamin A and its precursor, carotene, varies in accordance with the amount of vitamin E in the diet. It is postulated that vitamin E protects carotene and vitamin A against destruction in the intestine. It is possible that future research will discover more interrelationships of this type.

Vitamin A is now receiving prominent mention in the treatment of acetonemia. Acetonemia in cattle, ketosis in swine and pregnancy disease in sheep are akin to ketosis in humans. The clinical symptoms of acetonemia are varied but

may include loss of appetite, suspended rumination, reduced milk flow, constipation, unsteady gait loss of flesh, characteristic odor of the breath, impaired vision and an arched or swayed back. It occurs in many instances, under circumstances in which vitamin A deficiency is evident, but frequently little connection can be seen between the occurrence of acetonemia and A avitaminosis. The condition is characterized by low blood sugar and the presence of ketone bodies in the blood and urine. The ketone bodies indicate faulty fat metabolism. In the breakdown of fats in the body, a regular degradation proceeds by means of which the complex fat molecules are broken down into simpler compounds, finally producing acetoacetic acid which breaks down into acetic acid, thence to carbon dioxide and water which are easily expelled from the body. In the absence of adequate amounts of carbohydrates this degradation does not proceed normally, rather acetone instead of acetic acid is formed from the acetoacetic acid. Ketone bodies, acetone and related compounds, are formed whenever anything happens to interfere with the metabolism whereby carbohydrate becomes unavailable for the normal metabolism of fats.

It would seem probable that it is possible to differentiate between two types of acetonemia in cattle. The first type might be associated with a dietary history such as would be typified by the feeding of cottonseed and other oil meals and poor roughage. Vitamin A as well as carbohydrate might be lacking. In a second type the dietary might be fairly good except perhaps for body reserves. The heavy demands of flush milk production, complications of other disorders such as milk fever, the excessive demands for vitamin A for colostrum, and in all likelihood caloric imbalance may result in a depletion of the liver glycogen with accompanying low blood sugar. The carbohydrates necessary for the normal metabolism of fats are not available, hence acetonemia with the toxic acetone end products ensues.

In many instances the administration of vitamin A in massive dosages up to a million units, has restored the normal metabolism and alleviated the clinical symptoms. The reasons for this more or less miraculous effect of vitamin A are not so apparent. Although the mode of action of vitamin A in this connection is not completely understood there are certain facts concerning vitamin A which are noteworthy. The principal body storage of vitamin A as well as of glycogen occurs in the liver. It has been known for some years that vitamin A seems

to exert some non-specific functions upon fat and carbohydrate metabolism. Fat is necessary for the absorption and storage of vitamin A. In avitaminosis A the body deposits of fat decrease but are restored upon administration of vitamin A. The glycogen content of the liver decreases with low A intake and increases with excessive dosages of vitamin A.

Although the mode of action of vitamin A in alleviating acetonemia is not definitely known, it is probable that it acts by increasing the glycogen in the liver thereby making available the carbohydrates necessary for the normal metabolism of fats.

There has been considerable interest in the use of ascorbic acid (vitamin C) for the treatment of sterility in the bovine. The treatment has been either to inject ascorbic acid or to feed chloretone. The latter compound is one of a number of pure chemicals, all of which are nerve depressants, which have been reported to be effective in accelerating vitamin C synthesis in the rat and in the bovine. Early reports on the treatment of sterility in cattle were very promising. However, later reports are rather conflicting and in practice many animals have failed to respond to treatment with vitamin C.

Although vitamin E has been demonstrated to be essential for reproduction in experimental rats there is no clear-cut evidence to show any value of extra vitamin E for reproduction in dairy cattle and other large farm animals. Wheat germ oil has been used quite extensively in practice for this purpose without much apparent benefit in most instances, probably because the animals treated were not suffering from E avitaminosis.

Impaired reproductive ability accompanies many nutritive deficiencies. It has been recently reported that deficiencies of certain amino acids adversely affect reproduction in humans, and very promising clinical results are being obtained by the administration of certain amino acids in the treatment of human sterility. The clinical and pathological pictures involved in the whole question of sterility are gradually being elucidated and future developments will be awaited with much interest.

The use of vitamin D for the prevention of milk fever has been the subject of recent study. During milk fever the blood calcium, phosphorus and vitamin D are reduced. It is therefore rather logical to inquire whether or not vitamin D might not be effective in this disorder, especially in view of other physiological relationships in which vitamin D and calcium and phosphorus are closely associated. One million units of vitamin D daily have been fed experimentally for four weeks previous and one week after freshening. Results reported thus far have not been conclusive and it is apparent that the use of vitamin D under the conditions tried offers no assurance that milk fever will not occur. The possibility exists, however, that vitamin D may be found to play a role in this disorder.

Vitamin A concentrates are now on the market which are purported to be effective in increasing milk production. There has been a limited amount of research done with some of these materials without any very conclusive results. Under some conditions supplementary vitamin A has apparently increased milk production, but full acceptance of these products for increasing milk production must await further study.

During the war emergency unusual emphasis has been placed on protein in livestock nutrition. This is particularly true of protein derived from animal sources. It would now appear that limited supplies of protein have not resulted in the disaster which many persons predicted. This is true both for the production and the health of our animal population. It is logical to ask if in the future, we shall return to liberal protein feeding for growth and production. It is possible that past standards and opinions concerning protein should be revised somewhat. It is well established that irrespective of the source the main consideration is that certain necessary amino acids or their precursors are supplied. A popular or practical concept of protein includes not only the protein but also its adjuncts. Often the additional factors derived from certain sources of protein are very valuable and determine the choice of a source of protein. Dogs can now be raised and maintained on protein from vegetable sources, if all of the other factors that they require are also supplied. Thousands of pigs have been raised under conditions of inadequate protein nutrition according to past standards.

Many dairy rations now contain the pure chemical urea as a source of protein. There is considerable interest in ammoniated materials such as beet pulp and citrus pulp to be fed as a source of protein for cattle.

The ruminant is unique in its ability to use urea and similar products, as partial protein sources, when they are fed under proper conditions. It has been known for a long time that vitamin B complex factors are synthesized by micro-

organisms in the paunch of ruminants. The utilization of urea and other simple nitrogen compounds also apparently takes place through rumen synthesis. The microorganisms present in the rumen synthesize amino acids and protein from simple nitrogen compounds. The microorganisms then pass to the fourth stomach where they are digested and the resulting products of hydrolysis become available to the animal. The growth of these microorganisms depends on a suitable substrate being furnished in the diet of the animal, which should include a certain amount of protein, readily fermentable carbohydrate, and a source of nitrogen such as urea. Thus on timothy hay alone the utilization of urea is very low, but is improved by the addition of starchy feeds or molasses.

The amount of urea which should be included in a dairy feed is necessarily relatively low. Kidney damage may result from the feeding of too much urea. A safe level of feeding is approximately one to two per cent of the dry matter of the ration. The protein content of silages may be raised by the inclusion of 10 pounds of urea per ton of silage. Owing to its high content of nitrogen, small additions of urea provide relatively large amounts of protein. If large amounts of urea, of the order of 50 pounds per ton, are added to the silage, the resulting product may be very unpalatable owing to the production of free ammonia. Urea cannot be mixed with soybean meal if it contains the enzyme urease, owing to the production of free ammonia by the action of this enzyme on urea.

Urea is suitable as a partial source of protein for growing calves and for milch cows only if properly fed. A recent report claims that urea favors the increased synthesis of B vitamins by ruminants. It is probable that small amounts of urea will be continued to be used in the future, especially in some types of silage. The fact that it is useful only for ruminants and cannot be used by swine, horses, poultry and young calves and the fact that soybean urease and urea are incompatible are deterrents to its universal use.

There has been interest shown recently in the stimulation of milk production by the feeding of iodinated protein. Interest in this subject dates back some years to the discovery that the feeding of thyroxine or dessicated thyroid gland was effective in increasing milk production. Any practical value was nullified by the cost of this material. However, it has been found that a cheap compound which is a combination of casein and iodine has much the same physiological effect as thyroxine.

The feeding of 15 grams daily of protamone (iodinated casein) has been found to increase milk production five to 20 per cent and butterfat production 25 to 50 per cent in cows in the latter part of the lactation period. The pulse rate and respiration of the cows was also increased. This material is under the control of the Food and Drug Administration and is not available for general use.

More recently it has been found that thiourea and related compounds inhibit the formation of thyroxine by the thyroid gland. Since thyroidectomized steers have shown remarkable increases of weight, research has been instigated to determine means by which thiourea could possibly be used with fattening animals. Preliminary results with chickens, goats, lambs and calves indicate that feeding .25 to .75 gms. daily on a 100 lb. basis, for a limited time, improves the deposition of fat on the carcass. This work is in a very preliminary stage and the results will be followed with much interest.

A number of problems are of interest in the nutrition of swine. It is realized now that a ration which supports growth in pigs may still be deficient in vitamin A as evidenced by blindness and crooked backs in fairly mature pigs. Yellow corn cannot always be depended on to supply all of the needs of the growing pig for vitamin A. With respect to rickets and vitamin D the proper calcium-phosphorus ratio is of primary importance. Full advantage should be taken of all available sunshine. Supplemental vitamin D can be given in the form of cod liver oil or vitamin D yeast.

With respect to B vitamins, ordinary rations appear to supply all of the thiamine and riboflavin needed by pigs. The pig is a super conserver of thiamine and normally loads up its muscle tissues with this factor, so that pork is the outstanding source of thiamine among all foodstuffs. Niacin is required by the pig for growth and maintenance of a healthy gastrointestinal tract and may not be supplied in sufficient quantities in some practical swine rations. Most workers are willing to differentiate between infectious necrotic enteritis and the type of necro caused by a deficiency of niacin which is often referred to as swine pellagra. B vitamins, including niacin, have been found to be effective in promoting more rapid recovery in the treatment of enteritis with sulfa drugs. A characteristic goose-stepping sometimes seen in pigs and owing to nerve degeneration results from a deficiency of pantothenic acid and pyridoxine. It appears that practical rations are often deficient in

pantothenic acid, but in this type of disorder treatment with pantothenic acid is ineffective if the nerve degeneration is severe.

Undoubtedly faulty nutrition is one of the reasons that approximately one-third of the annual pig crop in this country dies before being weaned. It is fairly well established that the pig requires vitamin B complex factors which are unidentified to date. These factors are contained in liver and in yeast. They appear also to be present in alfalfa hay and in certain by-products of alcohol distillation. Relatively large amounts of alfalfa, up to 20 per cent, are now being recommended for swine.

Distillation by-products have come into increased use during the war period. Their value depends somewhat on the original material used in the alcohol fermentation. It should not be necessary to mention that for growing animals these products should be considered only as supplements to regular feedstuffs. As such they seem to be particularly valuable in furnishing some of the now unknown vitamin factors. They are for the most part deficient in vitamin A and when fed in combination with low grade roughage, as to fattening steers, supplementary vitamin A is required.

One may wonder how our animal population existed years ago when vitamins and new factors were unheard of. The answer is that although we may not realize it, livestock today are being handled under more and more unnatural conditions. Many pigs formerly rooted around in cow manure in the barnyard. Now we find that such manure contains factors required by the pig which are synthesized in the rumen of the cow. Many disorders of poultry were unknown when chickens had access to manure. Calves were formerly born in the spring when the dam was on new pasture containing plenty of vitamin A.

In those days the fields of chemotherapy, general pharmacology and nutrition were distinct and practically independent. Now a mutual independence is indicated between these previously specialized fields. The horizon is continually widening.

THE RAPIDITY OF THE STORAGE OF THIAMINE AND ITS RETENTION IN PORK MUSCLE

BY
J. W. PENCE, R. C. MILLER, R. A. DUTCHER
AND P. T. ZIEGLER

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INTENTIONAL SECOND EXPOSURE

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THE RAPIDITY OF THE STORAGE OF THIAMINE AND ITS RETENTION IN PORK MUSCLE[1]

J. W. Pence, R. C. Miller, R. A. Dutcher and P. T. Ziegler
Pennsylvania State College

In a previous report (Miller, *et al.*, 1943) it was shown that the thiamine content of pork muscle may be greatly increased by feeding a diet naturally rich in this vitamin or by feeding a ration supplemented with pure crystalline thiamine. These experiments involved feeding periods of from 99 to 140 days. Furthermore, it was shown that pork shoulder is consistently lower in thiamine content than pork loin. Preliminary observations were also reported for two pigs which were given 50 mg. of thiamine hydrochloride daily for 22 days. The pork-thiamine values for these pigs were of the same order as those for the pigs which had been on a high thamine regime for 99–140 days. These results indicated that the saturation point for thamine had been reached in the muscle tissues of the latter pigs. The data also indicated that the saturation of pork muscle by thiamine might occur quite rapidly. The experiments reported here were planned to give further information on these points.

Experimental Procedure

Thirty-six pigs of the Chester White, Duroc Jersey and Berkshire breeds, weighing approximately forty pounds each, were used in this experiment. These pigs were fed a basal ration composed of yellow corn, wheat, 60 percent digester tankage, ground alfalfa hay, limestone and salt, and which contained 1.2 mg. of thiamine per pound. The respective proportions of the ingredients of this ration were changed periodically, thereby reducing the protein content of the ration from 19.7 percent to 14.7 percent as the pigs grew. These changes did not affect the thiamine content of the ration. The pigs were kept in dry lots or indoors on cement floors, and did not have access to green feed. The experimental treatments were as follows: (1) Six pigs were fed the basal ration without supplementary thiamine for a period of 133 days. (2) Six pigs were fed the basal ration plus a daily supplement of 50 mg. of pure crystalline thiamine for a period of 155 days. (3) Six pigs were fed the basal ration for a period of 160 days. For 35 days, beginning 70 days previous to slaughter, they were given a daily supplement of 50 mg. of pure crystalline thiamine. Thus they did not receive thiamine during the 35 days immediately preceding slaughter. (4) Six pigs were fed the basal ration and

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a daily supplement of 50 mg. of pure crystalline thiamine for 35 days immediately preceding slaughter. Three other groups of three pigs each were given 50 mg. of supplementary thiamine daily for periods of 8, 15 and 22 days, respectively, immediately preceding slaughter. Two pigs received the supplementary thiamine for 29 days.

At the time of slaughter the pigs weighed approximately 190–210 pounds. Samples of the pork were analyzed in accordance with procedures described by Miller, *et al.* (1943).

Results and Discussion

The results are given in table 1. The pork-thiamine values for pigs fed the basal ration consisting of natural feedstuffs of average thiamine content correspond very closely with our previous results. They confirm previous observations to the effect that pork loin is richer in thiamine than pork shoulder, and that pork muscle is much higher in thiamine content than pork liver. The feed of these pigs contained 1.2 mg. of thiamine per pound and the average daily intake of thiamine was 5.3 mg.

The total average daily thiamine intake of the pigs which received a daily supplement of 50 mg. of pure crystalline thiamine for the entire feeding period of 155 days was approximately 11 times that of the pigs referred to above. The pork thiamine values for these pigs correspond closely with those previously published (Miller, *et al.*, 1943) for pigs on a high thiamine feeding regime, and are approximately twice as high as those for the pigs which received the usual amount of thiamine found in average feeds. These results emphasize the fact that the nutritional status of the pig is one of the factors determining the thiamine content of pork.

The pork-thiamine values for pigs which received 50 mg. of supplementary thiamine daily for 8, 15, 22 and 35 days, respectively, immediately preceding slaughter show that at this level of thiamine intake, the vitamin accumulates very rapidly in the muscle tissues. The thiamine content of the muscle tissues is approximately doubled in 35 days of high thiamine intake. The data for individuals show some variation between pigs on the same treatment, but this variation does not obscure the fact that the pig can store thiamine rapidly in muscle tissue. The pork-thiamine values for the pigs which received thiamine for 8 days show that there is a definite and rapid response to thiamine administration. It also appears that the muscle tissue becomes saturated with thiamine quite rapidly. The pork-thiamine values for the 35-day pigs and for the pigs given thiamine for 155 days correspond well with our previously-published values for pigs on a high thiamine regime.

The pork-thiamine values for pigs which received 50 mg. of thiamine daily for 35 days and then received no extra thiamine for the 35 days immediately preceding slaughter are relatively high and indicate that extra thiamine which is stored in pork-muscle tissue is retained by the tissue for some time after thiamine is withdrawn from the diet. Since these pigs gained in body weight quite rapidly during the last 35 days preceding slaughter, it may be doubtful if the pork-thiamine values actually represent any real loss of thiamine from the muscle tissue. This statement is made with full recognition of the fact that the tissues representing the body gains of pigs at this stage of their development, are comprised of a high percentage of fat.

TABLE 1. THE AVERAGE THIAMINE CONTENT OF PORK FROM PIGS WHICH RECEIVED A BASAL RATION WITHOUT SUPPLEMENTARY THIAMINE, AND WITH 50 MG. DAILY OF THIAMINE FOR VARIOUS PERIODS. (THIAMINE EXPRESSED AS MICROGRAMS PER GRAM OF FRESH TISSUE AND PER GRAM OF DRY MATTER)

No. of pigs	Shoulder		Middle of loin		Ham end of loin		Liver	
	Fresh basis	Dry matter basis	Fresh basis	Dry matter basis	Fresh basis	Dry matter basis	Fresh basis	Dry matter basis
Basal ration, no supplementary thiamine								
6	7.6±0.5	27.2±2.0	9.8±0.5	34.7±2.0	10.0±0.5	35.8±1.7	3.6±0.2	11.5±0.6
50 mg. thiamine daily for 8 days preceding slaughter								
3	10.2±0.5	36.7±1.6	11.6±0.4	42.6±0.6	12.6±0.2	47.0±2.0	3.2±0.2	10.3±0.4
50 mg. thiamine daily for 15 days preceding slaughter								
3	11.7±1.0	43.6±3.1	15.2±1.3	55.8±4.4	15.9±0.7	58.3±1.8	5.2±0.7	16.7±2.3
50 mg. thiamine daily for 22 days preceding slaughter								
3	13.8±1.5	44.7±3.8	17.4±0.6	56.5±3.4	17.1±0.5	58.0±5.2	5.5±0.4	17.4±1.0
50 mg. thiamine daily for 35 days preceding slaughter								
4	18.4±0.9	69.4±3.1	24.8±1.4	93.4±4.2	23.8±1.1	90.0±3.6	6.8±0.6	21.0±1.3
50 mg thiamine daily for 155 days preceding slaughter								
4	14.1±1.0	53.2±4.1	22.8±1.0	87.0±5.1	20.8±0.8	77.5±2.4	4.1±0.3	14.5±1.3
50 mg. thiamine daily for 35 days, then no supplementary thiamine for 35 days preceding slaughter								
6	14.8±0.6	56.0±2.6	19.6±0.8	73.1±3.5	20.4±1.1	77.0±5.0	4.8±0.4	15.2±1.1

It is impossible to determine accurately the retention of thiamine in a growing animal by tissue analyses. The alternative is to keep the animal on a

maintenance plane of nutrition for the desired length of time. However, the present results indicate strongly that the pig is probably different from other animal species which become depleted of thiamine stores quite rapidly.

This suggests that thiamine may be stored in pork muscle in a manner quite different from that in other species. Furthermore, thiamine may occur

TABLE 2. THE THIAMINE CONTENT OF THE PORK FROM PIGS WHOSE STORAGE OF THIAMINE WAS ATYPICAL (THIAMINE EXPRESSED AS MICROGRAMS PER GRAM OF FRESH TISSUE AND PER GRAM OF DRY MATTER)

Pig no.	Shoulder		Middle of loin		Ham end of loin		Liver	
	Fresh	Dry matter	Fresh	Dry matter	Fresh	Dry matter	Fresh	Dry matter
50 mg. thiamine daily for 155 days								
8	10.9	43.4	15.2	60.3	14.3	55.6	2.9	10.1
9	7.5	26.8	11.9	44.8	12.7	48.1	3.7	12.9
Ave.	9.2	35.1	13.6	52.6	13.5	51.9	3.3	11.5
50 mg. thiamine daily for 35 days previous to slaughter								
24	10.2	40.1	14.2	55.9	15.0	59.4	5.7	17.8
25	13.3	52.6	15.4	59.3	17.5	67.4	6.4	20.1
Ave.	11.8	46.4	14.8	57.6	16.3	63.4	6.1	19.0
50 mg. thiamine daily for 29 days previous to slaughter								
22	8.4	33.1	13.4	53.3	13.4	53.1	5.4	17.6
23	10.5	40.3	16.6	64.1	16.2	63.0	4.8	15.6
Ave.	9.5	36.7	15.0	58.7	14.8	58.1	5.1	16.

in pork muscle tissues in possibly several forms, and the forms in which it occurs in high and low concentrations may be different. Unpublished data, obtained by the authors, dealing with the stability of thiamine in pork, favor this hypothesis.

In some instances we have encountered pigs which exhibit a low storage response to thiamine feeding. Data for six such pigs are given in table 2. Having determined pork thiamine values for large numbers of pigs whose thiamine intakes were definitely known, the authors are of the opinion that, within the limits of normal variation, pigs give a uniform response to dietary thiamine, as shown by the thiamine content of the pork. The pigs, for which

data are given in table 2, are considered to be atypical with respect to thiamine storage in that they stored thiamine inefficiently or possessed low saturation capacities for this vitamin. Pigs numbers 9 and 22 stored little if any of the vitamin; low degrees of storage are shown by the other pigs. Experiments are planned to seek an explanation for these apparently anomalous cases.

Summary

Data are presented which show that thiamine can be stored at a rapid rate in pork muscle, and that these tissues can become saturated within 35 days or less. It is suggested that the nutritional status and the physiological status of the pig are factors which determine the thiamine content of pork. The pig differs from other animal species studied, with regard to the relatively slow rate of loss of stored thiamine after extra dietary thiamine has been withdrawn. Occasionally pigs are encountered which, for reasons still unexplained, are limited in their ability to store thiamine rapidly and efficiently.

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THE THIAMINE CONTENT OF PIG BLOOD*

BY JAMES W. PENCE, RUSSELL C. MILLER, R. ADAMS DUTCHER, AND WILLIAM T. S. THORP

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(Received for publication, February 8, 1945)

The normal concentration of thiamine in the blood of human subjects has been determined by several investigators. In most instances an average value of the order of 8.0 to 9.0 γ of thiamine per 100 ml. of whole blood has been found (1, 2). Goodhart and Sinclair (2) have reported a value of 5.7 γ per 100 ml. for the ox and the considerably higher value of 20.2 γ per 100 ml. for the pigeon. Schultz *et al.* (3) have found a value of 7.0 γ per 100 gm. of rat blood.

Since the thiamine content of the muscle tissue of the pig is normally much higher than that for the same tissue in other species, it is reasonable to expect that the thiamine content of pig blood would also be much higher. Similarly, because the thiamine content of muscle tissue in the pig may vary according to the amount of thiamine in the diet (4), the thiamine content of the blood should be related to the thiamine content of the diet or, more directly, to the thiamine content of the muscle tissue. Information on these points is given in this report.

EXPERIMENTAL

In order to determine the average normal concentration of thiamine, samples of blood were obtained from pigs receiving common herd rations. Some of the thiamine values represent pigs in the semifasting state, the blood samples having been taken at the time of slaughter from animals which had received no feed during the preceding 24 hours. In addition, non-fasting values were obtained from samples taken within 3 or 4 hours after the pigs had received a regular feeding. The blood samples were obtained from the anterior vena cava by the method of Carle and Dewhurst (5). Some of the blood samples were obtained from pigs fed ordinary fattening rations, the thiamine contents of which were not determined. However, most of the blood samples were taken from pigs receiving experimental diets of known thiamine content. In several instances pork samples were also obtained from these pigs.

In order to determine the effect of feeding relatively large amounts of

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thiamine upon the concentration of thiamine in the blood, daily individual doses of crystalline thiamine were administered orally to three groups of pigs. Each of a group of four pigs was given 25 mg. of thiamine for 7 days, and each pig in a similar group received 50 mg. of thiamine for the same period. Each of three pigs in a third group received 50 mg. of thiamine for a period of 11 days. Blood samples were taken before the thiamine feeding began and at regular intervals thereafter.

Thiamine was determined by a modification of the thiochrome method of Hennessy and Lewis (6). The method was modified in an attempt to reduce the hematin-catalyzed destruction of thiochrome by alkaline ferricyanide, as described by Owen, Weissmann, and Ferrebee (7). 5 ml. samples of whole, oxalated blood were pipetted into 40 ml. portions of 1 per cent acetic acid contained in 100 ml. volumetric flasks. The samples were heated at the temperature of boiling water for 10 minutes. After cooling, 0.3 gm. of taka-diastase in aqueous solution was added to each flask, and the samples were allowed to incubate at 45–50° for at least 2 hours. 40 ml. of 10 per cent trichloroacetic acid were then added to each flask, and the samples were allowed to stand for about 30 minutes. The pH was adjusted to about 4.5 with 2.5 M sodium acetate, the volume was made up to 100 ml., and the extracts were filtered. From this point the details of the published procedure were followed. Results were reproducible within ±15 per cent. A moderate decrease in the destruction of thiochrome was obtained with the modified procedure.

DISCUSSION

The results of the investigation are summarized in Table I and Fig. 1. Although a wide range of values was encountered in several of the groups, the standard errors of the mean values are well within the limits of the experimental error.

The normal concentration of thiamine in the blood of the pig appears to be about twice as high as that reported for human subjects. The mean value found for thirteen pigs in the semifasting state was 17 γ of thiamine per 100 ml. of whole blood. The mean blood thiamine value for nine non-fasting pigs was 21 γ per 100 ml. The difference between these values is statistically significant and suggests that the concentration of thiamine in the blood fluctuates during the progress of digestion in a manner similar to that of blood glucose and other substances.

In general, the concentration of thiamine in the blood becomes higher as the thiamine content of the diet is increased. In addition, there appears to be a rather direct relationship between the thiamine content of the muscle tissue of the pig and the concentration of thiamine in the blood. The data for Group 7, Table I, show clearly that when the tissue stores are large the blood thiamine level is high, even though the daily thiamine intake has been at an average level for some time. Similarly, in Group 8, although the daily thiamine intake was high, the tissue stores and the blood level of thiamine were only slightly above average. The relatively low tissue storage of thiamine in the latter animals is considered anomalous, as discussed elsewhere.[1]

TABLE I

Concentration of Thiamine in Normal Pig Blood and Its Relation to Thiamine Content of Diet and to Thiamine Content of Muscle Tissue

Group No.	Description of ration	Type of blood sample	No. of animals	Thiamine per 100 ml. whole blood			Average thiamine per gm. fresh loin tissue
				Range	Mean	Standard error	
				γ	γ	γ	γ
1	Herd ration of unknown thiamine content	Fasting	13	11–21	17	0.8	
2	" "	Non-fasting	5	16–21	19	0.8	
3	Herd ration containing 1.68 mg. thiamine per lb. feed	"	4	22–24	23	0.4	
4	Herd ration containing 1.75 mg. thiamine per lb. feed	Fasting	3*	16–20	18	1.2	10.7
5	Grain ration + legume pasture	"	2*	15–17	16	1.0	12.8
6	Herd ration + peanut skins or 50 mg. thiamine per day	"	4	25–35	29	2.3	19.4
7	Herd ration + 50 mg. thiamine for 35 days; then no extra thiamine for 35 days prior to slaughter	"	3	26–30	28	1.2	19.3
8	Herd ration + 50 mg. thiamine for 84 days	Non-fasting	3†	19–28	23	0.8	12.4

* Also included in Group 1.

† A total of ten determinations on samples of 3 succeeding days.

The influence of 7 days of feeding relatively large amounts of thiamine on the thiamine content of the blood is shown in Fig. 1. The level of thiamine in the blood increased rapidly during the period of administration of the vitamin, and decreased just as rapidly after the extra thiamine was discontinued. When the vitamin was fed for a period of 11 days (results not shown), the maximum blood level values attained were no higher than those reached within 5 to 7 days.

[1] Pence, J. W., Miller, R. C., Dutcher, R. A., and Ziegler, P. T., unpublished data.

There are several points of difference in the results obtained in this investigation and those obtained with human subjects. Benson *et al.* (1, 8), Youmans *et al.* (9), and other workers have found that the concentration of thiamine in human blood bears little relationship to the state of saturation of the tissues. Only in cases of severe thiamine deficiency does the blood level of thiamine fall below the normal range. In a deficient human subject, the administration of thiamine will cause the blood level to rise to normal values, but in mildly deficient or normal subjects an increase in the thiamine intake will not lead to higher blood thiamine values. Najjar and Holt (10), among others, report that when relatively large amounts of

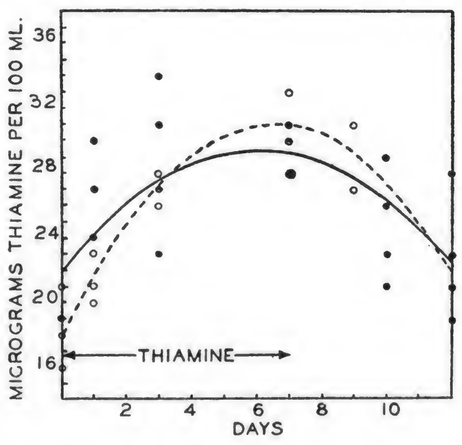

FIG. 1. The effect of feeding 25 (solid dots and line) and 50 mg. (clear circles and broken line) of crystalline thiamine daily for 7 days upon the thiamine content of pig blood.

thiamine are injected into human subjects the blood level rises to very high values, but returns to normal within a few hours.

The authors have shown[1] that the pig has the ability to accumulate large amounts of thiamine in muscle tissue quite rapidly. With an intake of 50 mg. of thiamine per day a maximum storage is obtained within 5 weeks, the thiamine content of these saturated tissues being nearly twice as high as that obtained from an average thiamine intake. Thus, if a high intake of thiamine is continued long enough to increase the storage of the vitamin in the muscle tissue materially, the concentration of thiamine in the blood will remain above normal even though the intake has been reduced (see the data for Group 7, Table I).

Within limitations, the concentration of thiamine in the blood may be used as a rough index of the storage of thiamine in the muscle tissue of the

pig. The storage of extra thiamine in the muscle tissue is indicated by an elevated blood thiamine value, provided that any substantial increase in the daily intake, immediately prior to sampling, has been of sufficient duration to have exerted its full effect upon the storage of this vitamin in the muscle tissue.

SUMMARY

The normal concentration of thiamine in the blood of pigs was found to be 17 and 21 γ per 100 ml. for semifasting and non-fasting pigs, respectively. These values are approximately twice as high as those reported for normal human subjects.

A rise in the blood thiamine level to approximately 30 γ per 100 ml. of whole blood occurred within a week when pure crystalline thiamine was fed in daily amounts of 25 or 50 mg.

The concentration of blood thiamine in the pig is directly related to the amount of thiamine in the diet and to the thiamine content of the muscle tissue.

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Apparatus for Determination of Rate of C

With Special Reference to Fa

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Department of Agricultural and Biological Chemistry, The Pennsylvania State

VARIOUS methods have been proposed for measuring the induction periods of fats (*1–5*). Most of these require a long period of time (at relatively low temperatures) or the continual presence of the investigator throughout the experiment, particularly if it is desirable to plot curves relating oxygen absorption to time.

This paper describes an apparatus which makes a continuous automatic record of the oxygen absorbed by a sample. No operator need be present after the experiment is started and tests may be run at elevated temperatures. An additional advantage over most previous methods which is inherent in this machine is the maintenance of a constant pressure in the reaction chamber irrespective of variations in atmospheric pressure.

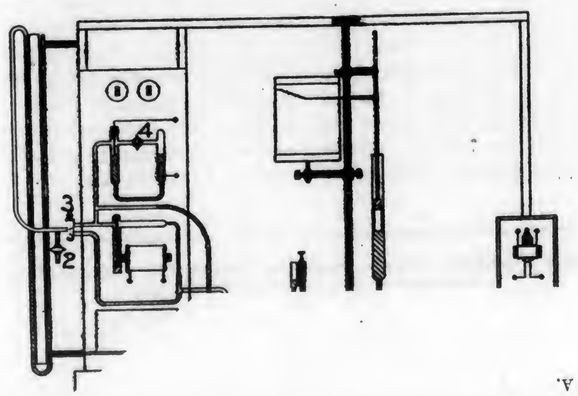
APPARATUS

The apparatus consists of a group of units.

CONSTANT-TEMPERATURE OVEN (Figure 1). The oven used in this setup was a Despatch baking oven modified by the introduction of a circulating fan and a mercury-over-toluene thermostat to maintain constant temperature.

PRESSURE REGULATOR. Construction is illustrated in Figure 2.

PRESSURE-CONTROL SYSTEM. This unit is designed to allow oxygen to flow from an oxygen reservoir into the absorption system under the influence of the pressure regulator without allowing any leakage either from or to the surrounding atmosphere. It is constructed from a large tire valve such as is used in a truck tire, a large electromagnet, etc. The details of construction are fully illustrated in Figure 3.



Figure

OXYGEN RESERVOI
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tube extending throug
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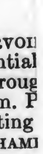

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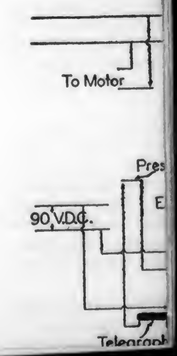

PRESENTED before the Division of Agricultural and Food Chemistry at the 99th Meeting of the AMERICAN CHEMICAL SOCIETY, Cincinnati, Ohio.

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(2) Greenbank and Holm, *Ibid.*, **17**, 625 (1925).
(3) Greenbank and Holm, IND. ENG. CHEM., ANAL. ED., **2**, 9 (1930)
(4) King, Roschen, and Irwin, *Oil & Soap*, **10**, 105 (1933).
(5) Triebold and Bailey, *Cereal Chem.*, **9**, 50 (1932).

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Apparatus for Determination of Rate of Oxygen Absorption

With Special Reference to Fats

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Department of Agricultural and Biological Chemistry, The Pennsylvania State College, State College, Pa.

VARIOUS methods have been proposed for measuring the induction periods of fats (*1–5*). Most of these require a long period of time (at relatively low temperatures) or the continual presence of the investigator throughout the experiment, particularly if it is desirable to plot curves relating oxygen absorption to time.

This paper describes an apparatus which makes a continuous automatic record of the oxygen absorbed by a sample. No operator need be present after the experiment is started and tests may be run at elevated temperatures. An additional advantage over most previous methods which is inherent in this machine is the maintenance of a constant pressure in the reaction chamber irrespective of variations in atmospheric pressure.

APPARATUS

The apparatus consists of a group of units.

CONSTANT-TEMPERATURE OVEN (Figure 1). The oven used in this setup was a Despatch baking oven modified by the introduction of a circulating fan and a mercury-over-toluene thermostat to maintain constant temperature.

PRESSURE REGULATOR. Construction is illustrated in Figure 2.

PRESSURE-CONTROL SYSTEM. This unit is designed to allow oxygen to flow from an oxygen reservoir into the absorption system under the influence of the pressure regulator without allowing any leakage either from or to the surrounding atmosphere. It is constructed from a large tire valve such as is used in a truck tire, a large electromagnet, etc. The details of construction are fully illustrated in Figure 3.

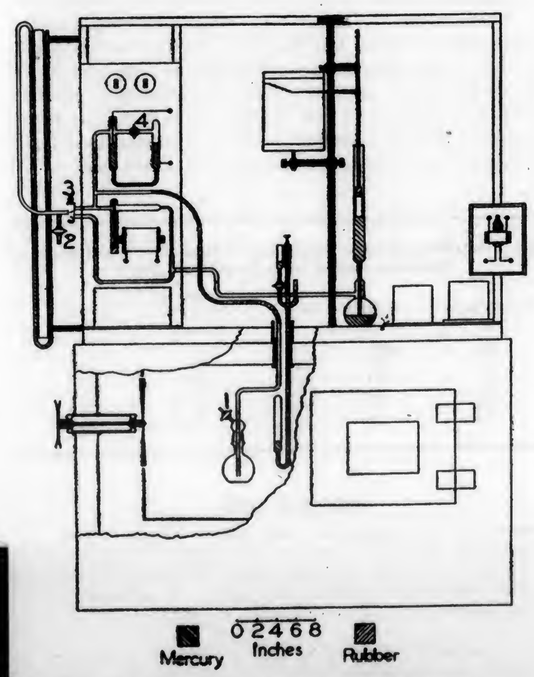

Figure 1. Diagram of Oxidation Apparatus

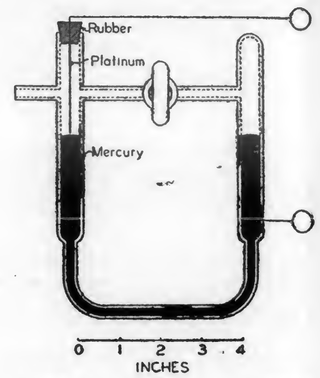

Figure 2. Diagram of Pressure Regulator

OXYGEN RESERVOIR AND RECORDING APPARATUS (Figure 1). This consists essentially of a Florence flask with a side arm and a tube extending through the neck of the flask. This tube is sealed to a 25 × 300 mm. Pyrex test tube in which the recording float operates. A rotating drum and pen complete the assembly.

ABSORPTION CHAMBER. Construction is clearly illustrated in Figure 1.

ELECTRICAL CIRCUITS. The wiring diagram given in Figure 4 shows the electrical circuits required.

PROCEDURE AND RESULTS

The oven is adjusted to the desired temperature by means of the thermostatic control.

A sample of fat (10 grams) is weighed into the absorption chamber and the absorption system is assembled with stopcock 1 open (Figure 1). Stopcock 2 is opened and stopcock 3 is turned so that the upper outlet is open. The absorption chamber is inserted into the oven and connected to the pressure-control system (Figure 1). Nitrogen is introduced through stopcock 2 and allowed to flow through the system for a few minutes. The flow of nitrogen is stopped and stopcocks 1 and 2 are closed. In order to allow the system to attain pressure equilibrium at atmospheric pressure, stopcock 2 is opened at intervals until no further change is apparent in the manometer, then stopcock 2 is opened momentarily while stopcock 4 is closed. Stopcock 3 is now turned so that the lower outlet is open, stopcock 2 is opened, and oxygen is introduced into the oxygen reservoir through stopcock 2 (it is advisable to flush the reservoir with oxygen several times before finally filling it).

The recording drum carrying a sheet of graph paper is put into position and the pressure in the oxygen reservoir is read from the manometer. Stopcock 3 is now turned so that the upper outlet is open and stopcock 1 is opened. Oxygen is introduced again through stopcock 2 and allowed to flush all the nitrogen out of the absorption chamber. The armature on the telegraphic relay is pulled over by hand to allow the recording pen to drop slightly when the oxygen flow is started. This records the start of the run.

After 2 to 3 minutes the flow of oxygen is stopped and stopcocks 1 and 2 are closed. The system is allowed to come to equilibrium and then slight suction is applied through stopcock 2 until the pressure control apparatus is activated. Stopcock 2 is closed while the pressure control valve is open; this valve should close automatically after a few seconds.

In general it is best to observe the manometer for 10 to 15 minutes after this last operation in order to be certain of pressure and temperature equilibrium. If no change is noted after this interval, stopcock 3 is closed and the system will automatically record the oxygen absorption.

If the pressure in the absorption system changes, suction is applied as before and the procedure is repeated from that point.

As the oxygen absorption proceeds and gas is taken up by the sample, the pressure in the absorption system is lowered, causing the mercury levels in the pressure controller to be shifted, so that the electrical circuit is completed through the telegraphic relay which then actuates the electromagnetic valve. The opening of this valve allows oxygen to flow from the reservoir into the absorption system until the pressure in the latter returns to its original condition, at which time the pressure-controller circuit opens again and the oxygen flow is cut off.

As gas is removed in this way from the reservoir it is replaced by mercury from the tube carrying the recording float. This allows the float to fall slightly and thus causes the pen to indicate a drop on the chart carried by the rotating drum. In the work reported here, the end of the induction period was taken at the first definite break in the curve. It is apparent that the number of breaks per unit of chart length is a measure of the rate of oxygen absorption.

After completion of a determination the quantity of oxygen which had been absorbed at the end of any time interval during the run may be calculated. If such figures are desired, the following data must be obtained:

Initial atmospheric pressure taken at the start of the run.
Manometer readings taken after the oxygen reservoir is filled at the start of the run.
Cross-sectional area of the Pyrex tube which carries the recording float.
Temperature in the region of the oxygen reservoir.

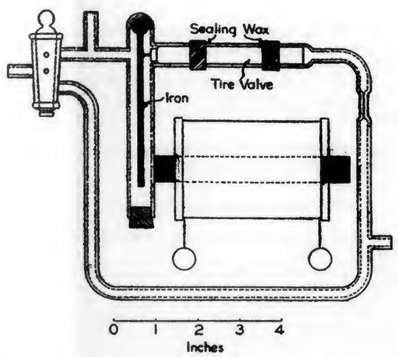

Figure 3. Diagram of Pressure-Control System

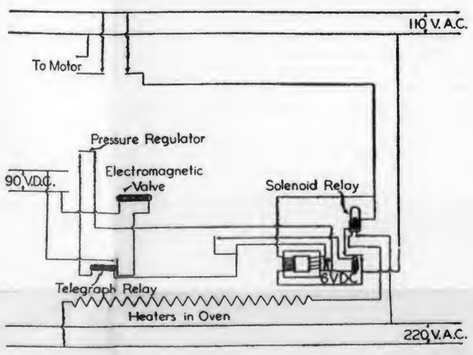

Figure 4. Diagram of Electrical Circuits Involved

* Rectifying circuit not shown

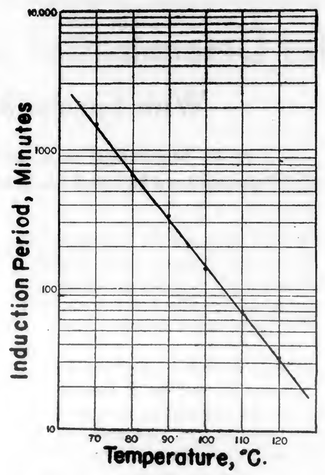

Figure 5. Relation between Temperature and Log of Induction Period of a Sample of Lard

The method of calculation is as follows: The total initial pressure in the oxygen reservoir is obtained by adding the initial atmospheric pressure to the difference in manometric levels at the start of the run. The change in pressure during the time interval under observation may be determined by measuring the pressure drop as indicated on the graph. This value is then subtracted from the total initial pressure to obtain the final pressure.

The drop recorded by the pen may be multiplied by the cross-sectional area of the tube to obtain the volume of oxygen absorbed and corrections may then be applied to this value, using the familiar equation

$$PV = NRT$$

and substituting the mean between initial and final pressures for P.

The accuracy of calculations based on these data depends on constancy of the temperature and atmospheric pressure. For this reason, only approximations can be obtained by this method. In the present machine production of gas during the reaction also conditions the accuracy of values obtained in this manner.

The apparatus herein described has been used in a study of the effect of temperature on the induction period of a sample of lard. The data given in Table I indicate that determinations can be made with a fairly high degree of accuracy as evidenced by the checks on duplicates. Figure 5 represents graphically the relation between temperature and the log of the induction period and indicates that the induction period is decreased by a factor of approximately 0.5 for each 9° increase in temperature.

Table I. Relation between Incubation Temperature and Length of Induction Period for a Sample of Lard

Temperature	Induction Period		Average of Replicates	Variation
° C.	*Minutes*		*Minutes*	%
70	1535	...	1535	...
80	636	666	651	4.6
90	325.9	346.6	336.3	6.2
100	138.5	138.7	138.6	0.1
110	67.3	69.9	68.6	3.8
120	32.1	33.4	32.8	4.0

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(2) Greenbank and Holm, *Ibid.*, 17, 625 (1925).
(3) Greenbank and Holm, IND. ENG. CHEM., ANAL. ED., 2, 9 (1930).
(4) King, Roschen, and Irwin, *Oil & Soap*, 10, 105 (1933).
(5) Triebold and Bailey, *Cereal Chem.*, 9, 50 (1932).

PRESENTED before the Division of Agricultural and Food Chemistry at the 99th Meeting of the AMERICAN CHEMICAL SOCIETY, Cincinnati, Ohio.

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Nutritive Value of Canned Foods

Determination of Ascorbic Acid of Fresh Green Peas

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PRELIMINARY studies conducted during the pea-canning season of 1943 indicated that ascorbic acid was lost in the preparation of a slurry in the Waring Blendor from a mixture of 300 grams of freshly vined peas and 150 grams of 3% metaphosphoric acid solution (batch A). This resulted in a final concentration of 1.0% metaphosphoric acid. However, this loss of ascorbic acid was not observed when similar slurries were prepared from the same batch of peas after they had been canned. A search of the literature failed to disclose the cause of the difficulty but it appeared that the destruction of ascorbic acid during and after blending raw peas may have been caused by active oxidative enzymes. As a result of this observation, studies were carried out to determine the conditions under which freshly vined peas must be handled in order to eliminate any loss of ascorbic acid during and after blending. To this end, representative samples of freshly vined peas, washed and unwashed, were blended with various concentrations of metaphosphoric acid in an atmosphere of nitrogen and in air.

These samples were selected originally from batches (300 pounds or more) of freshly vined peas. In order to obtain representative samples of peas from the batches, an initial sampling was effected in which 2-kg. portions were taken; 300 grams of this initial sample and 150 grams of metaphosphoric acid solution were blended. In the subsequent analyses reported here 200 grams of the initial sample and an equal weight of the metaphosphoric acid solution were blended in a Waring Blendor for 3 minutes. Nitrogen gas was supplied through a glass tube set in the cap of the Blendor bowl and directed downward below the surface of the mixture onto the knives of the bowl. A 10-gram aliquot of the slurry was transferred to a volumetric flask and made to volume with 3% metaphosphoric acid solution. This mixture was filtered (Whatman No. 12 paper) and an aliquot of the clear filtrate was titrated for reduced ascorbic acid with sodium 2,6-dichlorobenzenoneindophenol solution containing 40 mg. of dye per 100 ml. of water. The first pink color that persisted for 30 seconds was considered the end point.

The ascorbic acid values presented here are for the reduced form of the vitamin. No attempt was made to determine dehydroascorbic acid, since the methods which were available at that time for its determination did not lend themselves to routine analyses, especially under the conditions in which these field experiments were conducted.

Table I. Losses of Reduced Ascorbic Acid in Freshly-Vined Peas Blended with Metaphosphoric Acid in Air and in Nitrogen

Sample Designation, Batch	Treatment of Peas after Vining	Time after Blending	Final Concentration of Metaphosphoric Acid Blended in air	Final Concentration of Metaphosphoric Acid Blended in nitrogen	Ascorbic Acid Content of Peas	Ascorbic Acid Loss
		Min.	%	%	*Mg./100g.*	%
A	Washed	0	...	3.0	25.9	0.0
	Washed	10	...	3.0	25.9	0.0
A	Washed	0	...	1.0	20.1	22.4
	Washed	10	...	1.0	17.3	33.2
B-5	Washed	0	...	3.0	22.0	0.0
	Washed	60	...	3.0	22.0	0.0
	Washed	120	...	3.0	22.0	0.0
B-5	Washed	0	...	1.5	19.8	10.0
	Washed	60	...	1.5	17.0	22.7
	Washed	120	...	1.5	14.0	36.4
B-7	Washed	0	...	3.0	21.6	0.0
	Washed	60	...	3.0	21.6	0.0
	Washed	120	...	3.0	21.6	0.0
B-7	Washed	0	3.0	...	20.5	5.1
	Washed	60	3.0	...	20.5	5.1
	Washed	120	3.0	...	19.8	8.3
B-7	Washed	0	1.5	...	0.72	96.7
	Washed	60	1.5	...	0.58	97.3
C	Unwashed	0	...	3.0	31.3	0.0
	Unwashed	0	...	2.0	30.2	3.5
	Unwashed	0	...	1.0	24.5	21.7
C	Washed	0	...	3.0	28.1	0.0
	Washed	0	...	2.0	27.5	2.1
	Washed	0	...	1.0	24.8	11.7
D	Unwashed	0	...	3.0	32.8	0.0
	Unwashed	60	...	3.0	32.8	0.0
	Unwashed	0	3.0	...	32.8	0.0
	Unwashed	60	3.0	...	32.8	0.0
D	Washed	0	...	3.0	29.9	0.0
	Washed	60	...	3.0	29.9	0.0
	Washed	0	3.0	...	28.8	3.7
	Washed	60	3.0	...	28.4	5.0

The results of these studies, a summary of which is presented in Table I, indicate that a final concentration of 3% metaphosphoric acid, as produced by blending equal weights of fresh peas and a 6% metaphosphoric acid solution in an atmosphere of nitrogen, preserved the ascorbic acid for at least 1 hour. Later studies showed that ascorbic acid was retained without loss in a final concentration of 3% metaphosphoric acid for 2.5 hours. It appeared that a low final concentration of metaphosphoric acid (approximately 1%) was insufficient to inactivate the enzymes contained in fresh peas.

With an optimum concentration of metaphosphoric acid it is generally assumed that it is unnecessary to blend in an inert atmosphere. The authors' work indicates that, with a final concentration of 3% metaphosphoric acid, blending in an atmosphere of nitrogen does not appear to be absolutely essential in order to retain the ascorbic acid of raw peas. However, if the slurry is to be stored for any length of time and then reblended, small but significant losses of ascorbic acid occur when nitrogen is not employed.

Typical ascorbic acid values obtained after blending peas with various concentrations of metaphosphoric acid are shown in Table I, batches B-5, B-7, and C. Results with batches C and D show that freshly vined peas are higher in ascorbic acid before the vine and pod juices have been washed away. The unwashed peas were found to retain their ascorbic acid in a final concentration of 3% metaphosphoric acid regardless of the presence of nitrogen during the blending process. The latter observation was unexpected, in view of the fact that this experiment was performed primarily to test the inactivating power of a final concentration of 3% metaphosphoric acid on the enzymes of the vine and pod juices.

In supplementary studies, the use of oxalic acid in place of metaphosphoric acid was found less desirable, since oxalic acid solutions yielded turbid filtrates which are more difficult to titrate and usually give rise to higher ascorbic acid values.

SUMMARY

A final concentration of 3% metaphosphoric acid has been found optimum for blending raw peas in a Waring Blendor. The use of a nitrogen atmosphere for blending was advantageous in preventing oxidation, especially when the concentration of metaphosphoric acid was not optimum.

SUPPORTED in part by a grant from the National Canners Association–Can Manufacturers Institute Nutrition Program. This is the 12th of a series of papers dealing with the general subject, "The Nutritive Value of Canned Foods".

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UTILIZATION AND EXCRETION OF INGESTED ASCORBIC ACID BY THE DAIRY COW[1]

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AND S. I. BECHDEL
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INTRODUCTION

The influence of the diet of the dairy cow on the anti-scorbutic potency of the milk produced has occupied the interest of research workers in human nutrition and in dairy production since 1920 (6, 9, 10). The development of rapid chemical methods for the determination of ascorbic acid, notably the use of Tillman's reagent, sodium 2,6-dichlorobenzenoneindophenol, helped to substantiate the early findings of Hughes and co-workers (13) who found that the antiscorbutic potency of the milk produced was independent of the diet of the cow (12, 17, 21, 26). The amount of ascorbic acid in the milk has been reported to be influenced by breed and stage of lactation (11, 20, 28) and also by the season of the year (8, 11, 23) although the importance of the latter factor has been discounted by Kon (18).

The probable synthesis of ascorbic acid by the dairy cow has been suggested (12, 17, 18, 21, 22, 24, 25, 26) although no evidence is at hand to show where the synthesis takes place. Wendt (27) has claimed that ascorbic acid in cows' milk is synthesized by the udder parenchyma and that the amount of synthesis depends to some degree upon the condition of this organ.

Knight *et al.* (17) found that massive doses of ascorbic acid fed orally had no appreciable effect on the amount of ascorbic acid in the milk or blood plasma and only a slight effect on the amount of ascorbic acid excreted in the urine. Their findings relative to the rapid destruction of ascorbic acid in the rumen show this organ to be the original site of the loss of ingested ascorbic acid. However, Kendall and Chinn (15), working with rumen contents obtained from freshly slaughtered cattle, were unable to show decomposition of ascorbic acid in filtered rumen "liquid" *in vitro*.

While the available data do not point to a specific organ of synthesis they do substantiate the view that synthesis of ascorbic acid occurs in the bovine body. Most of the dietary experiments which bear on this subject have been of comparatively short duration. In the present investigation an attempt has been made to throw further light on the question. A Holstein cow was

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maintained on a low ascorbic acid ration for 15 months. During this period ascorbic acid determinations were made on the milk, the blood and the urine in order to follow changes in the ascorbic acid economy. Further studies were conducted to determine some of the factors affecting ascorbic acid destruction in the rumen.

EXPERIMENTAL

Methods and Apparatus

Ration. A representative four-year-old Holstein cow in the second month of her second lactation was selected as the experimental subject. During a period of one month this cow was continued on the herd ration while preliminary observations were made on the ascorbic acid content of the milk, the blood and the urine. Thereafter, an experimental diet of the following composition was fed:

	Per cent	*Per cent protein*
Casein	5.0	4.5
Dry yeast[2]	4.0	2.0
Hominy feed	65.5	7.2
Cornstarch	13.5	.....
Corn gluten meal	10.0	4.3
Salt mixture	2.0	.....
	100.0	18.0

One pound of this feed mixture was fed for every 3½ pounds of milk produced, but the daily ration was never reduced below 10 pounds. The daily ration was mixed with five pounds of dried beet pulp and divided into three portions which were fed at 6 A.M., 1 P.M. and 9 P.M., respectively. It was found necessary to supplement the basal ration daily with three pounds of oat straw or heat-treated low grade timothy hay as a further source of roughage. At weekly intervals, 10 ml. of shark liver oil (25,000 I.U. per g.) were fed as a source of vitamin A.

Blood Plasma. Blood was drawn from the jugular vein into an oxalated tube containing sufficient paraffin oil to protect the surface of the blood from contact with air. The tube was cooled immediately and protected from light. Plasma was separated by centrifuging in an angle centrifuge. Blood-plasma ascorbic acid was determined as follows: Five ml. of plasma and 10 ml. of 10 per cent metaphosphoric acid were pipetted into a centrifuge tube containing 5 ml. of distilled water. The tube was centrifuged in order to facilitate a sharper separation of the precipitated proteins, and aliquots of the supernatant metaphosphoric acid extract were taken for ascorbic acid determinations. An Evelyn photoelectric colorimeter was used with the

[2] 25% of the yeast was high potency vitamin D yeast (Fleischmann's type, 7–F, 3,200,000 U.S.P. units per pound).

buffered dye solution suggested by Mindlin and Butler (19) and corrections for turbidity and color were made according to Bessey (2).

Milk. Milk was collected in an ice-cooled brown bottle containing carbon dioxide. The apparatus, described by Knight *et al.* (16), consisted of a brown bottle fitted with a three-holed rubber stopper. In this stopper were placed; 1) a glass adapter with rubber tubing to fit over the teat, 2) a Bunsen valve to allow carbon dioxide, which was displaced by the incoming milk, to escape, and 3) a clamped rubber tube which would admit a 10-ml. pipette for the removal of aliquots of the milk sample. An aliquot of milk was pipetted into an equal volume of 10 per cent metaphosphoric acid solution. After shaking to break up aggregates of precipitated protein the mixture was titrated directly with indophenol dye solution to a pink end-point which persisted for 30 seconds.

Urine. The 24-hours urine sample was collected in a large carboy containing sufficient acetic acid to give a final concentration of about 5 per cent acetic acid. The urinal employed in the collection of urine and the method of collection have been described by Knight *et al.* (17). Three or four times during the collection, carbon dioxide gas was employed to flush air from the collection bottle. About 500 ml. of the collected urine were withdrawn under carbon dioxide pressure into an ice-cooled brown bottle containing carbon dioxide gas. Aliquots of the urine-acetic acid solution were analyzed for ascorbic acid, usually within an hour after obtaining the sample. A 5-ml. aliquot of the urine-acetic acid solution was pipetted into a colorimeter tube placed in the Evelyn photoelectric colorimeter and 5 ml. of buffered dye solution were added in three seconds. Galvanometer readings were taken at 5-second intervals for 30 seconds and these values were extrapolated to determine the zero-second reading.

Rumen Liquid. Two months after parturition a rumen fistula operation was performed as described by Bechdel *et al.* (1). Samples of rumen contents were obtained through the rumen fistula with the aid of a special long-handled dipper. *In vivo* samples were filtered through cheesecloth into a measured amount of 10 per cent metaphosphoric acid. Samples for *in vitro* studies were kept warm and immediately transferred to the *in vitro* apparatus which will be described later. Ascorbic acid in the rumen liquid was determined photoelectrically by a variation of the colorimetric method in which the unreacted dye was extracted with zylene as suggested by Bukatsch (4). The validity of the method depends on the assumption that the ascorbic acid reacts instantaneously with the dye and that the unreacted dye in the acid form is quantitatively removed by xylene from the aqueous acid phase. Once the residual dye is in solution in the xylene it is stable for several hours, thereby eliminating the necessity of taking readings immediately.

The procedure employed was as follows: 5 ml. of the unknown solution, containing 0–100 micrograms ascorbic acid in 5 per cent metaphosphoric

acid solution, were added to 12 ml. of a 2 mg. per cent dye solution, buffered to pH 6.8 according to Mindlin and Butler (19), in a 50-ml. centrifuge tube. To this were added immediately 20 ml. of xylene. The tube was stoppered and shaken vigorously for 15 seconds to remove the unreacted dye from the aqueous layer into the xylene. The tube was centrifuged and at least 10 ml. of the xylene were decanted into a colorimeter tube and read in the Evelyn photoelectric colorimeter which had been set at 100 with xylene in a matched tube. The photometric density L_s of the unknown solution was taken from the chart of $G - L$ values[3] and the L_b of a blank tube was likewise found. The difference L_b–L_s is a measure of the photometric density L of the sample which may be calculated from the formula (19):

$$C = K\ (\log G_s - \log G_b) \quad \text{or} \quad C = K\ (L_b - L_s)$$

The value of K was determined using known quantities of ascorbic acid, ranging in concentration from 0–100 micrograms per 5 ml. of five per cent metaphosphoric acid solution. It was found by plotting the galvanometer readings against concentration on semi-logarithmic paper that the relationship was represented by a straight line.

RESULTS

Blood Plasma. Blood plasma ascorbic acid determinations by the method described above gave values ranging from 0.28 to 0.59 mg. per 100 ml. plasma. This is well within the range of values reported by Bortree *et al.* (3). They reported a range of 0.11 to 0.80 mg. per 100 ml. blood plasma for 24 normal cows.

Milk. Results observed during the study of changes in milk production and ascorbic acid secretion in the milk, during most of one lactation period, are presented in graphical form in figure 1. The gradual decrease in the excretion of ascorbic acid in the milk paralleled the decline in milk production. The course of the ascorbic acid decline appeared to bear no relationship to the ascorbic acid intake.

Urine. The urinary ascorbic acid excretion was generally unpredictable except that the ingestion of large amounts of ascorbic acid appeared to increase the rate of excretion temporarily. Over a four-month period the urinary excretion averaged 57 mg. of ascorbic acid daily while the cow was subsisting on the low ascorbic acid ration.

In figure 2 the average hourly excretions of urinary ascorbic acid during the daytime and at night are compared. Except in one instance, the 184th night, the daytime hourly excretion was distinctly higher than the corresponding night excretion in terms of milligrams ascorbic acid excreted per hour. It should be noted that a large amount of ascorbic acid was fed during the 183rd day and apparently was carried over into the night collection.

Rumen Studies with Crystalline Ascorbic Acid. In figure 3 the rate of

[3] G is the galvanometer reading and $L = 2 - \log G$.

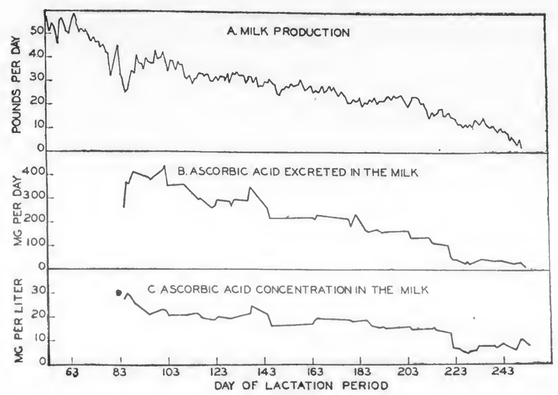

FIG. 1

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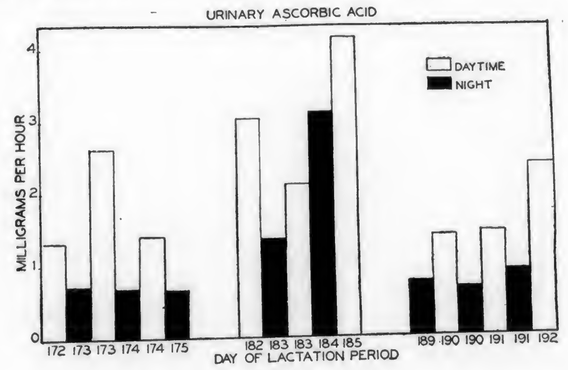

FIG. 2

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UTILIZATION AND EXCRETION OF INGESTED ASCORBIC ACID BY THE DAIRY COW[1]

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INTRODUCTION

The influence of the diet of the dairy cow on the anti-scorbutic potency of the milk produced has occupied the interest of research workers in human nutrition and in dairy production since 1920 (6, 9, 10). The development of rapid chemical methods for the determination of ascorbic acid, notably the use of Tillman's reagent, sodium 2,6-dichlorobenzenoneindophenol, helped to substantiate the early findings of Hughes and co-workers (13) who found that the antiscorbutic potency of the milk produced was independent of the diet of the cow (12, 17, 21, 26). The amount of ascorbic acid in the milk has been reported to be influenced by breed and stage of lactation (11, 20, 28) and also by the season of the year (8, 11, 23) although the importance of the latter factor has been discounted by Kon (18).

The probable synthesis of ascorbic acid by the dairy cow has been suggested (12, 17, 18, 21, 22, 24, 25, 26) although no evidence is at hand to show where the synthesis takes place. Wendt (27) has claimed that ascorbic acid in cows' milk is synthesized by the udder parenchyma and that the amount of synthesis depends to some degree upon the condition of this organ.

Knight *et al.* (17) found that massive doses of ascorbic acid fed orally had no appreciable effect on the amount of ascorbic acid in the milk or blood plasma and only a slight effect on the amount of ascorbic acid excreted in the urine. Their findings relative to the rapid destruction of ascorbic acid in the rumen show this organ to be the original site of the loss of ingested ascorbic acid. However, Kendall and Chinn (15), working with rumen contents obtained from freshly slaughtered cattle, were unable to show decomposition of ascorbic acid in filtered rumen "liquid" *in vitro*.

While the available data do not point to a specific organ of synthesis they do substantiate the view that synthesis of ascorbic acid occurs in the bovine body. Most of the dietary experiments which bear on this subject have been of comparatively short duration. In the present investigation an attempt has been made to throw further light on the question. A Holstein cow was

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maintained on a low ascorbic acid ration for 15 months. During this period ascorbic acid determinations were made on the milk, the blood and the urine in order to follow changes in the ascorbic acid economy. Further studies were conducted to determine some of the factors affecting ascorbic acid destruction in the rumen.

EXPERIMENTAL

Methods and Apparatus

Ration. A representative four-year-old Holstein cow in the second month of her second lactation was selected as the experimental subject. During a period of one month this cow was continued on the herd ration while preliminary observations were made on the ascorbic acid content of the milk, the blood and the urine. Thereafter, an experimental diet of the following composition was fed:

	Per cent	*Per cent protein*
Casein	5.0	4.5
Dry yeast[2]	4.0	2.0
Hominy feed	65.5	7.2
Cornstarch	13.5	.....
Corn gluten meal	10.0	4.3
Salt mixture	2.0	.....
	100.0	18.0

One pound of this feed mixture was fed for every 3½ pounds of milk produced, but the daily ration was never reduced below 10 pounds. The daily ration was mixed with five pounds of dried beet pulp and divided into three portions which were fed at 6 A.M., 1 P.M. and 9 P.M., respectively. It was found necessary to supplement the basal ration daily with three pounds of oat straw or heat-treated low grade timothy hay as a further source of roughage. At weekly intervals, 10 ml. of shark liver oil (25,000 I.U. per g.) were fed as a source of vitamin A.

Blood Plasma. Blood was drawn from the jugular vein into an oxalated tube containing sufficient paraffin oil to protect the surface of the blood from contact with air. The tube was cooled immediately and protected from light. Plasma was separated by centrifuging in an angle centrifuge. Blood-plasma ascorbic acid was determined as follows: Five ml. of plasma and 10 ml. of 10 per cent metaphosphoric acid were pipetted into a centrifuge tube containing 5 ml. of distilled water. The tube was centrifuged in order to facilitate a sharper separation of the precipitated proteins, and aliquots of the supernatant metaphosphoric acid extract were taken for ascorbic acid determinations. An Evelyn photoelectric colorimeter was used with the

[2] 25% of the yeast was high potency vitamin D yeast (Fleischmann's type, 7–F, 3,200,000 U.S.P. units per pound).

buffered dye solution suggested by Mindlin and Butler (19) and corrections for turbidity and color were made according to Bessey (2).

Milk. Milk was collected in an ice-cooled brown bottle containing carbon dioxide. The apparatus, described by Knight *et al.* (16), consisted of a brown bottle fitted with a three-holed rubber stopper. In this stopper were placed; 1) a glass adapter with rubber tubing to fit over the teat, 2) a Bunsen valve to allow carbon dioxide, which was displaced by the incoming milk, to escape, and 3) a clamped rubber tube which would admit a 10-ml. pipette for the removal of aliquots of the milk sample. An aliquot of milk was pipetted into an equal volume of 10 per cent metaphosphoric acid solution. After shaking to break up aggregates of precipitated protein the mixture was titrated directly with indophenol dye solution to a pink end-point which persisted for 30 seconds.

Urine. The 24-hours urine sample was collected in a large carboy containing sufficient acetic acid to give a final concentration of about 5 per cent acetic acid. The urinal employed in the collection of urine and the method of collection have been described by Knight *et al.* (17). Three or four times during the collection, carbon dioxide gas was employed to flush air from the collection bottle. About 500 ml. of the collected urine were withdrawn under carbon dioxide pressure into an ice-cooled brown bottle containing carbon dioxide gas. Aliquots of the urine-acetic acid solution were analyzed for ascorbic acid, usually within an hour after obtaining the sample. A 5-ml. aliquot of the urine-acetic acid solution was pipetted into a colorimeter tube placed in the Evelyn photoelectric colorimeter and 5 ml. of buffered dye solution were added in three seconds. Galvanometer readings were taken at 5-second intervals for 30 seconds and these values were extrapolated to determine the zero-second reading.

Rumen Liquid. Two months after parturition a rumen fistula operation was performed as described by Bechdel *et al.* (1). Samples of rumen contents were obtained through the rumen fistula with the aid of a special long-handled dipper. *In vivo* samples were filtered through cheesecloth into a measured amount of 10 per cent metaphosphoric acid. Samples for *in vitro* studies were kept warm and immediately transferred to the *in vitro* apparatus which will be described later. Ascorbic acid in the rumen liquid was determined photoelectrically by a variation of the colorimetric method in which the unreacted dye was extracted with zylene as suggested by Bukatsch (4). The validity of the method depends on the assumption that the ascorbic acid reacts instantaneously with the dye and that the unreacted dye in the acid form is quantitatively removed by xylene from the aqueous acid phase. Once the residual dye is in solution in the xylene it is stable for several hours, thereby eliminating the necessity of taking readings immediately.

The procedure employed was as follows: 5 ml. of the unknown solution, containing 0–100 micrograms ascorbic acid in 5 per cent metaphosphoric

acid solution, were added to 12 ml. of a 2 mg. per cent dye solution, buffered to pH 6.8 according to Mindlin and Butler (19), in a 50-ml. centrifuge tube. To this were added immediately 20 ml. of xylene. The tube was stoppered and shaken vigorously for 15 seconds to remove the unreacted dye from the aqueous layer into the xylene. The tube was centrifuged and at least 10 ml. of the xylene were decanted into a colorimeter tube and read in the Evelyn photoelectric colorimeter which had been set at 100 with xylene in a matched tube. The photometric density L_s of the unknown solution was taken from the chart of $G - L$ values[3] and the L_b of a blank tube was likewise found. The difference L_b–L_s is a measure of the photometric density L of the sample which may be calculated from the formula (19):

$$C = K\ (\log G_s - \log G_b) \quad \text{or} \quad C = K\ (L_b - L_s)$$

The value of K was determined using known quantities of ascorbic acid, ranging in concentration from 0–100 micrograms per 5 ml. of five per cent metaphosphoric acid solution. It was found by plotting the galvanometer readings against concentration on semi-logarithmic paper that the relationship was represented by a straight line.

RESULTS

Blood Plasma. Blood plasma ascorbic acid determinations by the method described above gave values ranging from 0.28 to 0.59 mg. per 100 ml. plasma. This is well within the range of values reported by Bortree *et al.* (3). They reported a range of 0.11 to 0.80 mg. per 100 ml. blood plasma for 24 normal cows.

Milk. Results observed during the study of changes in milk production and ascorbic acid secretion in the milk, during most of one lactation period, are presented in graphical form in figure 1. The gradual decrease in the excretion of ascorbic acid in the milk paralleled the decline in milk production. The course of the ascorbic acid decline appeared to bear no relationship to the ascorbic acid intake.

Urine. The urinary ascorbic acid excretion was generally unpredictable except that the ingestion of large amounts of ascorbic acid appeared to increase the rate of excretion temporarily. Over a four-month period the urinary excretion averaged 57 mg. of ascorbic acid daily while the cow was subsisting on the low ascorbic acid ration.

In figure 2 the average hourly excretions of urinary ascorbic acid during the daytime and at night are compared. Except in one instance, the 184th night, the daytime hourly excretion was distinctly higher than the corresponding night excretion in terms of milligrams ascorbic acid excreted per hour. It should be noted that a large amount of ascorbic acid was fed during the 183rd day and apparently was carried over into the night collection.

Rumen Studies with Crystalline Ascorbic Acid. In figure 3 the rate of

[3] G is the galvanometer reading and $L = 2 - \log G$.

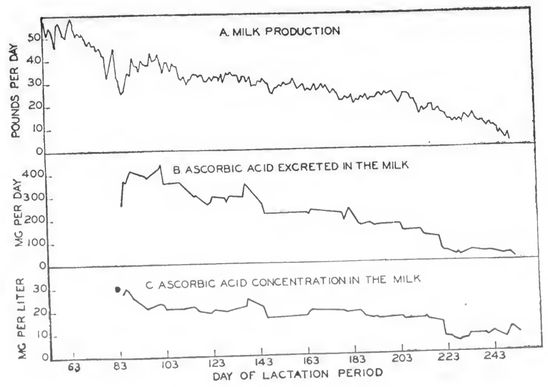

FIG. 1

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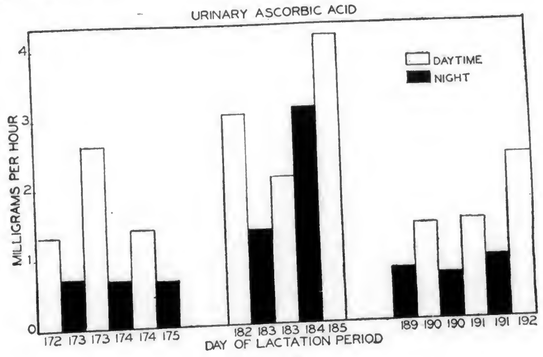

FIG. 2

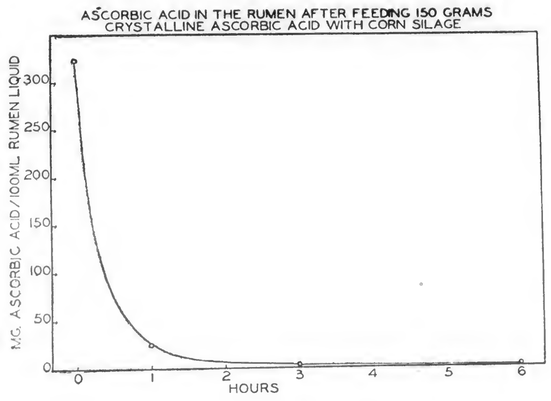

FIG. 3

Effect of Microorganisms. Kendall and Chinn (15) reported that ascorbic acid-fermenting microorganisms are less numerous in the rumen than the non-ascorbic-acid-fermenting types. In order to determine the role of microorganisms in the destruction of ascorbic acid in the rumen, an *in vitro* study was performed in which a sample of rumen contents was sterilized by autoclaving and then incubated in the *in vitro* apparatus. The *in vitro* apparatus was essentially a constant temperature (40° C.) water bath in which the sample of rumen contents was suspended in a rubber balloon attached to a hinged arm. The end of the arm opposite the hinge was lifted and dropped every 20 seconds by means of a revolving eccentric in order to simulate the peristaltic action of the rumen. Figure 5 shows the slower rate of *in vitro*

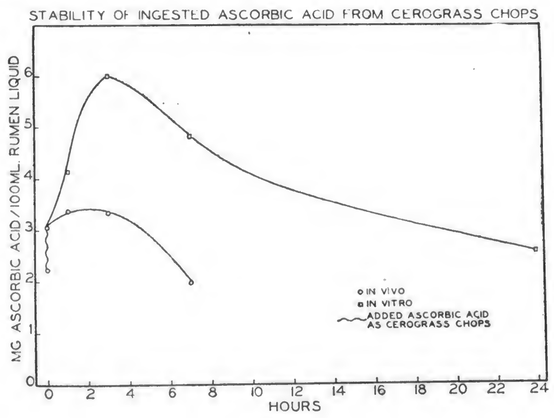

FIG. 4

TABLE 1

A comparison of in vivo with in vitro destruction of ascorbic acid in rumen "liquid" added in the form of "Cerograss Chops"

Time	Additions	Ascorbic acid in rumen "liquid"	pH of rumen "liquid"
hours		*mg. per 100 ml.*	
		in vivo	
Start	None	2.23	.....
Start	*Cerograss chops*	3.05	5.95
1		3.36	5.70
3		3.34	5.69
7		1.98	6.41
		in vitro	
Start	*Cerograss chops*	3.05	5.95
1		4.14	5.59
3		6.00	5.30
7		4.82	5.15
24		2.56	5.02

destruction of ascorbic acid in the sterilized rumen contents as compared with the unsterilized rumen contents.

The *in vivo* studies were continued but in a different manner. The rumen was completely emptied, flushed with warm water and again emptied after which the cow was allowed to partake of the low ascorbic acid ration *ad libitum.* An *in vivo* study of the stability of the ascorbic acid was performed as well as an *in vitro* study. The results of this study are shown in figure 6 titled *without inoculum.* Under the above conditions, there appeared to be little if any tendency for the destruction of ascorbic acid. When this experiment was repeated with the exception that an inoculum of two gallons of the fermenting rumen contents was returned to the rumen, results were obtained as shown in figure 6 titled *with inoculum.* In the latter case there was a definite tendency for the destruction of ascorbic acid.

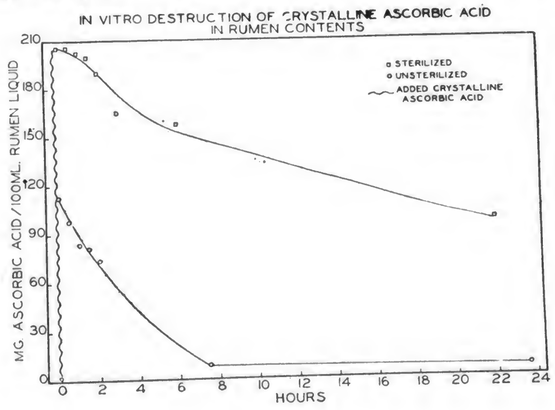

FIG. 5

DISCUSSION

These experiments indicate that the dairy cow is able to function normally for a period of at least 8 months on a ration containing small amounts of ascorbic acid (less than 100 mg. daily). In a four-month period from the 85th to the 224th day of one lactation period the average daily ascorbic acid intake was 99.7 mg. In this period the ascorbic acid excretion in the milk averaged 258 mg. daily and the urinary excretion averaged 57 mg. daily. The excretion of ascorbic acid in the milk alone greatly exceeded the intake. Our studies as well as those of Knight *et al.* (17) show that ascorbic acid is rapidly destroyed during rumen fermentation. It is quite evident therefore that the synthesis of ascorbic acid by the dairy cow must occur in the tissues and/or in some part of the digestive tract other than the rumen.

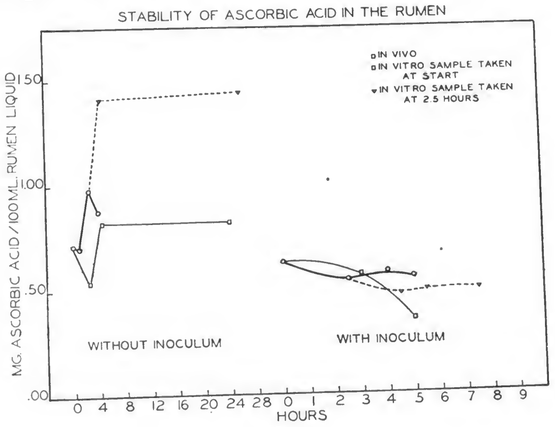
STABILITY OF ASCORBIC ACID IN THE RUMEN
IN VIVO
IN VITRO SAMPLE TAKEN AT START
IN VITRO SAMPLE TAKEN AT 2.5 HOURS
MG. ASCORBIC ACID / 100 ML. RUMEN LIQUID
1.50
1.00
.50
.00
WITHOUT INOCULUM
WITH INOCULUM
0 4 8 12 16 20 24 28 0 1 2 3 4 5 6 7 8 9
HOURS

FIG. 6

It is possible that a ration devoid of ascorbic acid, however impractical, would produce different results. It may be argued that such a procedure is necessary to completely satisfy the hypothesis that the dairy cow is capable of synthesizing ascorbic acid for all of her metabolic requirements.

The concentration of ascorbic acid in the blood plasma has served as a criterion of the state of ascorbic acid nutrition (21). Bortree *et al.* (3) have reported marked variations with animals, also variation from day to day and from hour to hour. Wallis (26) found average values ranging from 0.270 to 0.432 mg. per 100 ml. of plasma, but the variations were not consistent with the vitamin C intakes.

Every effort was made to complete the blood plasma analyses as soon as possible after venipuncture although as pointed out by Kassan and Roe (14) ascorbic acid in whole blood is stable when the red cells are preserved intact. After separation of the plasma care must be exercised to avoid oxidizing

ascorbic acid. In the method of Butler and Cushman (5), in which the deproteinization and the filtration steps are carried out in an atmosphere of carbon monoxide, the possibilities of oxidizing the ascorbic acid are eliminated. A comparison of the latter method with the method reported in this paper was favorable. However, the method employing carbon monoxide is much more time consuming.

The apparent lack of influence of the dietary intake of ascorbic acid on the ascorbic acid potency of the milk produced is borne out by the data presented in figure 1. This fact was recently pointed out by Wallis (25, 26). This worker found that one group of cows on pasture showed the lowest ascorbic acid concentration in the milk while a second group, which was fed an experimental ration low in ascorbic acid, showed a larger amount of ascorbic acid in the milk and was surpassed only by a third group which was fed alfalfa hay and grain.

During the time the cow was on the low-ascorbic acid ration she gave birth to a normal male calf. A sample of colostrum taken less than 12 hours after parturition contained 17.1 mg. ascorbic acid per liter. Three days later the milk contained 16.6 mg. per liter and two weeks later 16.5 mg. per liter. On the basis of these values it appears that the colostrum was not appreciably higher in ascorbic acid content than the milk. These findings, while limited to one animal, are of interest owing to the fact that the impression is quite universal that colostrum is characterized by a higher concentration of ascorbic acid than is found in normal milk. According to Rasmussen *et al.* (20) "This high ascorbic acid content of colostrum was believed to indicate a certain degree of storage of this substance by the cow during the preparturition period." If this assumption were correct, it would follow that a storage of ascorbic acid failed to occur in this instance presumably owing to a scarcity of ascorbic acid in the ration.

On the other hand Kon and Watson (18) concluded that the vitamin C content of colostrum is only slightly higher than that of milk. In their experiment with four cows, three showed a vitamin C content of colostrum higher than the average for the milk of the herd while the fourth had definitely less. To these workers it appeared that the difference between colostrum and milk is much smaller in the case of vitamin C than it is in the case of vitamin A and carotene. On the basis of these observations, no significance should be attached to the "low" ascorbic acid content of the colostrum.

The use of a photoelectric colorimetric method in the determination of ascorbic acid in cows' urine was an attempt to overcome, at least in part, the effect of other reducing substances which decolorize the dye. It was assumed that the reaction of ascorbic acid with the dye was almost immediate, whereas the reactions of non-ascorbic-acid-reducing substances present in cows' urine were slower and continued for some time. The method is admittedly empirical but Evelyn *et al.* (7) state: "The errors are less serious

than those of the ordinary visual titration which may often amount to several hundred per cent.''

The urinary excretion of ascorbic acid appeared to follow no general trend and varied from 14.1 to 97.8 milligrams per 24 hours.

Differences in the amount of ascorbic acid excreted in the daytime as compared with the night excretion may be a result of: 1) greater intake of water during the warmer daytime as compared with the cooler night, 2) greater metabolic acitivity during the daytime or 3) assuming that the excretion of ascorbic acid occurs immediately following feeding, a larger excretion in the daytime, because two of the three feedings were made during the daytime collection.

In spite of the rapid destruction of relatively large amounts of crystalline ascorbic acid there always remained a very small but apparently constant amount (about 0.5 mg. per 100 ml. rumen ''liquid'') of ascorbic acid in the rumen.

SUMMARY

A Holstein cow was maintained in normal physical condition for a period of 15 months on a ration containing very little ascorbic acid. During this period a normal male calf was born. Blood plasma ascorbic acid values ranged from 0.28 to 0.59 mg. per 100 ml. but usually fluctuated between 0.30 and 0. 45 mg. per 100 ml. As the lactation period progressed the total daily ascorbic acid excretion in the milk diminished in a manner paralleling the decrease in milk volume. During this time the urinary ascorbic acid excretion appeared to be erratic with values ranging from 14.1 to 97.8 mg. per 24 hours. The ingestion of large amounts of ascorbic acid increased the urinary excretion of the vitamin very slightly and then only temporarily. The rate of urinary excretion of the vitamin appeared to be greater during the daytime than during the night.

In vivo and *in vitro* rumen studies, made possible with the aid of a rumen fistula, showed that crystalline ascorbic acid in amounts of 150 g. was rapidly destroyed in the rumen. Ascorbic acid supplied in a natural form as *Cerograss Chops,* was also destroyed in the rumen but at a slower rate owing, presumably, to the slower dissolution of ascorbic acid from the natural grasses. These studies also indicated that the destruction of ascorbic acid in the rumen was a result, at least in part, of the action of microorganisms.

The results of these experiments may be summarized, in support of the probable synthesis of ascorbic acid by the dairy cow, as follows: 1) ascorbic acid either in the crystalline form or as a part of a natural product (*Cerograss Chops*) is destroyed rapidly in the rumen; 2) the apparent independence of the cow for dietary ascorbic acid is reflected by normal concentrations of ascorbic acid in the blood and milk in spite of a low ascorbic acid intake over a prolonged period of time; and finally, 3) the ingested intake of ascorbic acid on this lowascorbic acid ration is exceeded by the combined output of the vitamin in the milk and the urine.

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Fate of Lead Arsenate Spray During Grape Juice Packing

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PRESENT practice of applying insecticide and fungicide sprays to grapes is almost universal. The quantities of chemicals required for pest control are sufficiently large in most instances so that a considerable spray residue remains on the surface of the fruit at harvest, and may in some cases constitute a potential public health hazard unless removed in some manner. Federal and state laws and regulations specify amounts which legally may be present on fruits or in fruit products, if these are to be offered to the public.

In the past, the grape grower has had little difficulty in meeting these legal tolerances, because the number of poison sprays required in most instances was small. However, with an increase in the infestation of such pests as the grape berry moth, there is a tendency to increase the number of sprays containing calcium or lead arsenate, and to apply these sprays later in the growing season. These practices naturally result in higher spray deposits at harvest time.

Most of the Concord grape crop in the Lake Erie belt is processed into juice or grape preserves. The question of the amount of spray residue present on the grapes therefore becomes of considerable concern to the grape growers and the manufacturers of these products. The processors, in particular, are interested in knowing the fate of these spray deposits during the manufacturing process.

The work reported in this paper was planned to answer these questions. Specifically, it is a study of the fate of arsenic and lead present on the surface of juice grapes during processing into the final product, grape juice.

Typical cluster of Concord grapes as used in the production of grape juice.

Experimental Procedure

This experiment was carried out during 1943 and 1944, and involves grapes grown in two vineyards. In 1943 and 1944 samples were processed from a plot receiving a relatively heavy spray application, designated as (A) for identification, while in 1944, another vineyard (B), receiving fewer sprays, was included. The spray schedule for these two vineyards is given in Table 1. It is to be noted that vineyard A received a total of four sprays containing lead arsenate, the last being applied on July 27, 1943, and on July 12, 1944. Vineyard B received but two sprays containing lead arsenate, the second on June 30, 1944. The additional sprays containing fixed nicotine and summer oil applied later to both vineyards served to protect the earlier arsenate deposits from weathering.

Samples of approximately 60 pounds of grapes were collected on September 28, 1943 (vineyard A) and on September 29, 1944 (vineyard A and B). These grapes were removed to the processing plant, weighed accurately, and stemmed by hand. The grapes were then crushed, warmed to 140° F. and transferred to the press. Records were taken on the amounts of free-run, mid-press, and final-press juice, and samples collected for analysis. The pomace was weighed and sampled for analysis. After sampling, all of the juice from each vineyard was thoroughly mixed, and one-gallon samples were bottled, pasteurized, and placed in storage. One lot of juice was stored at cellar temperature (approximately 65° F) while another lot was stored in a refrigerated room at 26° F. One-gallon samples were withdrawn after approximately 45 days, while the remainder was allowed to remain in storage for an additional 45 days. At the end of the storage period the argols were filtered off, and samples of the filtrate and argols preserved for chemical analysis.

All samples were digested with nitric and sulfuric acid, arsenic determined by the bromate method (1), and lead by the sulfide method (2).

Results

A record of the weights of the various fractions of the grapes as produced

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The authors express their appreciation for the assistance rendered by the officials of the Welch Grape Juice Company in whose plant the experimental juice pressings were made.

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(1) Official and Tentative Methods of Analysis, Association of Official Agricultural Chemists, Fifth Edition, 1940, pp. 393-4.

(2) A Simplified Method for the Rapid Determination of Lead Residues on Apples. D. E. H. Frear and D. E. Haley. Penna. Agr. Exp. Station Bulletin 304, 1934.

during the juicing process is given in Table 2. In general, there was very close agreement between the three samples represented in this table. It appears that very nearly 70 per cent of the original weight of the grapes was recovered as juice. The weight of pomace average 18.0 per cent of the original weight of grapes, while just under 10 per cent of the original weight was lost—presumably on the filter cloth, etc. This loss would probably be much less in a large-scale operation.

The distribution of lead originally present on the grapes is given in Table 3. The data in this table again show a good agreement between the three samples processed. The original amounts of lead present showed considerable variation, the lowest being 414.1 milligrams (0.114 grains per pound), while the highest was 635.6 mg. (0.165 grain per pound). All samples exceeded the legal tolerance for lead residues, 0.050 grain per pound.

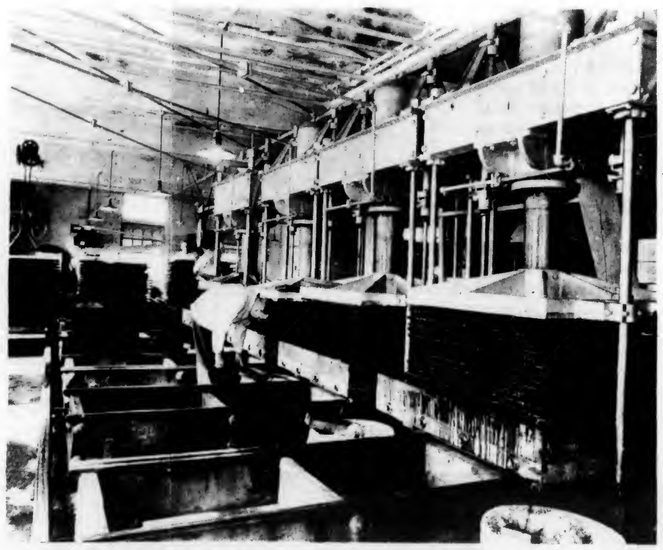
During the processing of the grapes into juice, more than one-third (35.8 per cent) of the lead residues was discarded with the stems. Nearly half of the lead originally present on the grapes remained with the pomace, and only 17.1 per cent of the original lead residue was present in the final expressed juice. Interesting comparisons may be made between the weights of the various fractions (Table 2) and the lead distribution (Table 3). The stems, amounting to only 3.1 per cent of the original weight of the picked grapes, carried 35.4 per cent of the lead. This is logical, since at the time of the early sprays, the area of the stems was relatively high in proportion to the area of the berries, so that the former received a fairly high proportion of the spray deposit. Lead, being relatively insoluble, remains on the grape skins, so that much of the element on the outer surface of the grapes remains there, and passes along with the pomace in a disproportionate ratio to the weight of the pomace (47.7 *vs.* 18.0 per cent). The small amount of the original lead in the juice is particularly striking: 70.3 per cent of the original weight of the grapes contained but 17.1 per cent of the lead. Calculated another way, the final juice contained only 0.032 grain of lead per pound, on the average.

Table 1

Composition (amounts per 100 gallons) and time of application of sprays. Concord juice grapes, 1943-1944

Vineyard A Composition		Date 1943	Date 1944	Vineyard B Composition		Date 1944
			Pre-blossom			
Lead arsenate	3.0 lbs.	June 10	June 9	Omitted		
Copper sulfate	2.0 lbs.					
Lime	3.0 lbs.					
Summer oil	3.0 qts.					
			Post-blossom			
Lead arsenate	4.0 lbs.	June 22	June 19	Lead arsenate	3.0 lbs.	June 22
Copper sulfate	2.0 lbs.			Copper sulfate	2.0 lbs.	
Lime	3.0 lbs.			Lime	3.0 lbs.	
Summer oil	3.0 qts.			Summer oil	3.0 qts.	
Nic. sulfate 40%	0.75 pt.			Nic. sulfate 40%	0.5 pt.	
			7-10 day spray			
Lead arsenate	3.0 lbs.		June 27	Same as post-blossom spray		June 30
Copper sulfate	2.0 lbs.					
Lime	3.0 lbs.					
Summer oil	3.0 qts.					
Nic. sulfate 40%	0.75 pt.					
			Before touching			
Same as 7-10 day spray		July 27*	July 12	Black leaf 155	3.0 lbs.	July 7
				Summer oil	3.0 qts.	
			Final			
Black leaf 155	3.0 lbs.	Aug. 27	Aug. 18	Same as before touching spray		Aug. 3

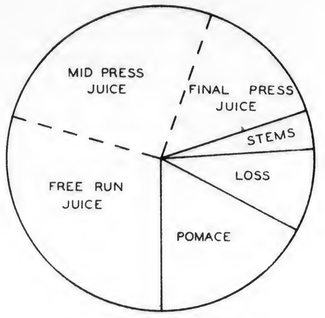
*This spray was designated as a "late touching", rather than "before touching".



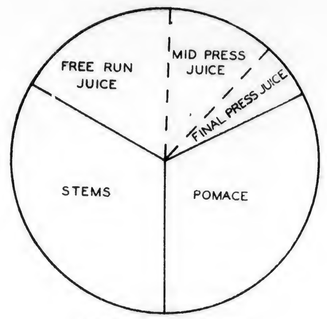
Grapes after being washed, stemmed and heated, are brought to this battery of hydraulic presses, where the juice is extracted. Photo of Welch Grape Juice Co.

The comparable figures for arsenic distribution are given in Table 4. The amounts of arsenic found on the original grapes (expressed as As_2O_3) closely paralleled the amounts of lead found, and approach the 2:1 ratio in lead arsenate. This indicates that the weathering process had not seriously broken down the spray deposit chemically. The amounts of arsenic on the original grape clusters varied from 248.3 mg. to 370.5 mg., or from 0.068 to 0.095 grain per pound. The legal tolerance for As_2O_3 is 0.025 grain per pound.

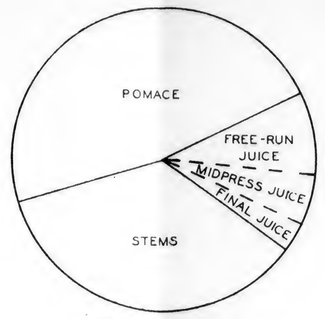
Approximately one-third of the arsenic, like the lead, was present on the grape stems, although less arsenic remained with the pomace, and consequently more passed into the juice. An average of 34.7 per cent of the arsenic originally present on the grape clusters ultimately found its way into the juice. In the samples considered here this amounted to a mean value of 0.036 grain per pound of juice. In all probability the presence of this amount of arsenic in the juice indicates that the organic acids of the juice have reacted with the lead arsenate, forming a soluble compound of arsenic, and, as has been shown, an insoluble compound of lead.



WEIGHT DISTRIBUTION
Figure 1



ARSENIC DISTRIBUTION
Figure 2



LEAD DISTRIBUTION
Figure 3

The data given in Tables 2, 3, and 4 are presented graphically in Figures 1, 2, and 3. In these figures it is possible to see at a glance the distribution of original weight, lead, and arsenic in the various fractions. The area of the circle in each case represents the original grapes.

Storage Studies

The juice, stored in one gallon lots at two different temperatures for approximately 45 and 90 days, was separated from the precipitated argols and both portions analyzed for lead and arsenic. Considerable difficulty was experienced in making the analyses of the argols, and the results are subject to a slightly higher degree of error than those reported in the previous tables. For this reason all of the analytical results for the stored samples were averaged, and the means only are presented in Table 5. The slight discrepancies in this table are attributable to the analytical error, which is magnified when dealing with small amounts present in some of the samples.

In the main, however, the conclusions of this storage study are quite clear. It is apparent that during storage at 65° F. 16.1 per cent of the lead was precipitated with the argols after approximately 90 days, while at 26° F. 25.4 per cent of the same element was precipitated. These percentages are calculated on the lead present in the original *juice*. With arsenic, only a negligible amount was precipitated during storage, the maximum being 4.0 per cent. The data did not permit any explanation of this phenomenon. It is possible, however, that lead, being a basic element, combines chemically with the tartaric acid in the juice, and is precipitated along with the other

Table 2

Amounts and proportions of various fractions of grapes involved in the production of juice

	Vineyard A 1943 Kilograms	1943 Per cent of total	Vineyard A 1944 Kilograms	1944 Per cent of total	Vineyard B 1944 Kilograms	1944 Per cent of total	Mean percentage distribution*
Original grapes	27.7	100.0	27.1	100.0	25.5	100.0	100.0
Berries	26.8	96.8	26.2	96.7	24.8	97.3	96.9
Stems	0.9	3.2	0.9	3.3	0.7	2.7	3.1
Juice	19.2	69.3	19.4	71.9	17.8	69.8	70.3
Free run	(11.5)	(41.6)	(6.1)	(22.6)	(5.7)	(22.4)	(28.9)
First press	(5.8)	(20.8)	(8.6)	(31.9)	(6.8)	(26.7)	(26.5)
Second press	(1.9)	(6.9)	(4.7)	(17.4)	(5.3)	(20.8)	(15.0)
Pomace	4.1	14.8	5.3	19.6	5.0	19.6	18.0
Loss	3.5	12.6	2.3	8.5	2.0	7.8	9.6

*Arithmetic means of appropriate entries in columns 2, 4 and 6.

Table 3

Distribution of lead in the various fractions of grapes involved in the production of juice

	Vineyard A 1943 Milligrams	1943 Per cent of total	Vineyard A 1944 Milligrams	1944 Per cent of total	Vineyard B 1944 Milligrams	1944 Per cent of total	Mean percentage distribution*
Original grapes	465.5	100.0	635.6	100.0	414.1	100.0	100.0
Berries	288.9	62.1	423.4	66.6	265.0	64.0	64.2
Stems	176.6	37.9	212.2	33.4	149.1	36.0	35.8
Juice	64.2	13.8	122.6	19.3	75.7	18.2	17.1
Free run	(46.1)	(9.9)	(55.8)	(8.8)	(34.8)	(8.4)	(9.0)
First press	(14.7)	(3.2)	(51.5)	(8.1)	(28.7)	(6.9)	(6.1)
Second press	(3.4)	(0.7)	(15.3)	(2.4)	(11.9)	(2.9)	(2.0)
Pomace	224.7	48.3	302.6	47.6	195.2	47.1	47.7

*Arithmetic means of appropriate entries in columns 2, 4 and 6.

Table 4

Distribution of arsenic in the various fractions of grapes involved in the production of juice

	Vineyard A 1943 Milligrams	1943 Per cent of total	Vineyard A 1944 Milligrams	1944 Per cent of total	Vineyard B 1944 Milligrams	1944 Per cent of total	Mean percentage distribution*
Original grapes	252.7	100.0	370.5	100.0	248.3	100.0	100.0
Berries	173.7	68.8	254.0	68.6	152.2	61.3	66.2
Stems	78.9	31.2	116.5	31.4	96.1	38.7	33.8
Juice	114.0	45.1	124.1	33.5	63.5	25.5	34.7
Free run	(79.3)	(31.4)	(45.4)	(12.3)	(27.3)	(11.0)	(18.2)
First press	(28.9)	(11.4)	(56.0)	(15.1)	(20.2)	(8.1)	(11.5)
Second press	(5.8)	(2.3)	(22.7)	(6.1)	(16.0)	(6.4)	(4.9)
Pomace	59.7	23.6	133.1	35.9	93.4	37.6	32.4

*Arithmetic means of appropriate entries in columns 2, 4 and 6.

Table 5
Average amounts of lead and arsenic present in grape juice and argols after storage at two temperatures

	65° F storage			26° F storage		
	Milligrams	Per cent	Grains per pound	Milligrams	Per cent	Grains per pound
Lead in original juice	92.8		0.034	92.8		0.034
Lead in juice stored 45 days	80.8		0.030	72.2		0.027
Lead in juice stored 90 days	76.2		0.028	70.8		0.026
Lead in argols after 45 days	10.9	11.7		21.9	23.6	
Lead in argols after 90 days	14.9	16.1		23.6	25.4	
Arsenic in original juice	85.2		0.032	85.2		0.032
Arsenic in juice after 45 days	82.2		0.031	85.5		0.032
Arsenic in juice after 90 days	78.9		0.029	79.0		0.029
Arsenic in argols after 45 days	1.7	2.0		3.4	4.0	
Arsenic in argols after 90 days	2.0	2.3		3.1	3.6	

tartrates in the argols, while arsenic, being in a soluble form, is not so precipitated.

Summary and Conclusions

Samples of grapes sprayed with lead arsenate were processed into juice on a semi-commercial scale during 1943 and 1944. All samples carried spray residue loads in excess of the legal limits for lead and arsenic. A study of the fate of these two elements during the processing indicated that, although 70.3 per cent of the original weight of the grapes was recovered as juice, only 17.1 per cent of the lead and 34.7 per cent of the arsenic was found in this fraction.

A considerable portion of the lead present in the juice was precipitated with the argols on storage. This amounted to 25.4 per cent of the original lead present in the juice when stored at 26° F. for 90 days. Only a negligible amount of arsenic was precipitated during storage. It appears that this latter element presents more of a problem in the processing of grape juice than does lead.

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Nutritive Value of Canned Foods

Comparison of Vitamin Values Obtained by Different Methods of Assay

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Six representative foods—corn, peas, carrots, tomatoes, salmon, and ham—were prepared as purees or pastes to ensure uniformity of composition, processed by commercial methods, and packed in tin containers. The resulting products were assayed for ascorbic acid, carotene (or vitamin A), thiamine, riboflavin, pantothenic acid, and niacin by several collaborators, using accepted methods of vitamin assay. The data obtained indicate the need for more extensive collaborative studies, using representative samples of unquestionable uniformity, prepared in quantity from the same source and stored under the same conditions prior to assay, stable vitamin reference standards, and methods in which all details of procedure and quality of reagents are prescribed.

THE general plan of a comprehensive investigation concerning the nutritive value of canned foods in which a number of educational institutions and industrial research laboratories are participating has been outlined (*1*). Since the estimation of the vitamin content of canned meats, fish, fruits, and vegetables constitutes an important phase of this investigation, the present report concerns primarily a comparison of vitamin values as obtained by applying existing vitamin assay methods to typical canned foods. Many investigators in this field are aware that vitamin assay methods which yield excellent results with one type of foodstuff may be unsatisfactory when applied to another food material. Consequently, the authors have endeavored to compare the results obtained through the use of recognized biological, microbiological, chemico-physical, and spectrographic methods of assay from standardized representative canned foods. In addition, they have sent samples of the same foods to several other laboratories, which have generously collaborated in furnishing additional data relative to the application of some of these methods to canned foods.

FOOD MATERIALS USED

All canned foods used in this investigation were furnished through the courtesy of the National Canners Association–Can Manufacturers Institute Nutrition Program Executive Committee. The products consisted of four canned vegetables, a canned fish, and a canned meat.

The specific products employed were yellow corn, peas, carrots, tomatoes, salmon, and ham. In order to ensure uniformity of sample the four canned vegetables were pureed and recanned in 8-ounce tins. The canned salmon was made into a thick paste and recanned in 12-ounce tins. The canned meat (chopped ham) was processed in 12-ounce tins and represented a commercial pack especially well mixed, so as to minimize can-to-can variations. On being received at the laboratory, all samples were stored at 30° F. until used for assay purposes or sent to collaborating laboratories.

In addition to the assays conducted in the authors' laboratory, samples (1 to 4 cans) of one or more of the six canned products were sent to investigators associated with 21 laboratories located in various parts of the country, accompanied by the request that (*a*) the product or products be assayed in duplicate or triplicate determinations by the method or methods commonly employed in the collaborating laboratory, (*b*) the method of assay be specified in each instance, and (*c*) the vitamin content of each product be expressed in terms of U.S.P. units or as milligrams of vitamin per 100 grams of can contents.

METHODS OF ASSAY

In general, the methods of vitamin assay employed were essentially those indicated below, with certain collaborators resorting to minor modifications of these procedures as required by materials and equipment at hand.

BIOLOGICAL METHODS. The U.S.P. method was employed in the estimation of carotene and vitamin A, the resulting growth responses being compared with that produced by known amounts of pure β-carotene and the usual U.S.P. reference cod liver oil, respectively. Thiamine was determined by the U.S.P. method and also by the rat-growth method. Riboflavin and pantothenic acid were determined by rat-growth methods. In each assay procedure where rat-growth technique was employed, a basal diet was used which was complete in all known dietary essentials with the exception of the vitamin under consideration. To ensure against any possible complication due to inadequate intakes of vitamins, liberal quantities of all of the known essential vitamins, with the exception of the vitamin to be assayed, were added in highly purified form to the basal diet. All rats used in assays

Table I. Vitamin Content of Specially Prepared Canned Foods
(Milligrams per 100 grams of can contents)

Laboratory No.	Method of Assay	Pureed Corn	Pureed Peas	Pureed Carrots	Pureed Tomatoes	Salmon Paste	Chopped Ham
	Reduced Ascorbic Acid						
1	Indophenol[a]	8.98	3.19	2.04	20.54	0.76	0.47
3	Indophenol[b]	5.00	4.70	5.70	21.70	...	...
4	Indophenol[b]	10.10	3.10	3.60	21.20	...	...
5	Indophenol[b]	11.00	4.00	3.00	26.00	...	...
5	Indophenol[c]	9.00	3.00	...	22.00	...	...
6	Indophenol[b]	...	...	...	20.25	...	...
7	Indophenol[b]	9.50	3.60	1.70	19.20	...	...
9	Indophenol[b]	10.08	3.95	2.46	24.33	...	...
	Carotene						
1	Biological[d,e]	0.21	0.47	7.30	1.91	...	...
1	Photocolorimetric	0.17	0.44	5.75	1.57	...	...
2	Photocolorimetric	0.14	0.20	5.80	1.80	...	...
3	Photocolorimetric	0.33	0.24	8.21	1.21	...	...
4	Photocolorimetric	0.15	0.40	9.79	1.79	...	...
5	Photocolorimetric	0.21	0.18	6.00	1.26	...	...
9	Photocolorimetric	0.38	0.20	8.19	...	...	...
12	Photocolorimetric	0.08	0.29	7.20	1.60	...	...
	Thiamine						
1	Biological (curative)	0.068	0.181	0.037	0.110	0.021	0.737
1	Thiochrome	0.057	0.150	0.027	0.102	0.007	0.522
2	Thiochrome	0.045	0.101	0.020	0.075	0.006	0.240
3	Thiochrome	0.046	0.115	0.021	0.084	...	...
4	Thiochrome	...	...	0.037	...	...	...
5	Thiochrome	0.050	0.120	0.030	0.090	0.002	0.420
6	Thiochrome	...	0.146	...	0.104	...	...
7	Thiochrome	0.055	0.159	0.025	0.094	...	0.475
8	Thiochrome	...	...	...	...	...	0.573
11	Thiochrome	0.051	0.131	0.022	0.095	0.016	

[a] Determined colorimetrically.
[b] Determined titrimetrically.
[c] Determined potentiometrically.
[d] Pure β-carotene used as standard.
[e] When assayed by U.S.P. biological method using U.S.P. reference cod liver oil as standard, corn, peas, carrots, and tomatoes yielded 236, 700, 8130, and 2190 U.S.P. units, respectively, per 100 grams.

involving growth response measurements were subjected to the usual depletion period previous to being placed on test. Growth responses were recorded over a 28-day period.

During the course of the biological assays, a fresh can of food was used at each feeding period. In the authors' laboratory, the microbiological assays and the assays made by the chemico-physical methods were made on composite samples composed of the entire contents of six cans of the respective products. Some collaborating laboratories employed the contents of a single can for their assays, while other laboratories used composite samples made from the contents of several cans.

MICROBIOLOGICAL METHODS. Riboflavin was determined by the method of Snell and Strong (*6*), pantothenic acid by the method of Pennington, Snell, and Williams (*5*), and niacin by the method of Snell and Wright (*7*).

CHEMICO-PHYSICAL METHODS. Since preliminary tests had indicated that the samples involved contained only relatively small amounts of dehydroascorbic acid, only reduced ascorbic acid was determined and this was done by the Morell indophenol photoelectric colorimeter method (*4*). Carotene was extracted and purified by the method of Moore and Ely (*3*) and its absorption at 450 mμ determined by means of a Beckman quartz spectrograph, and thiamine and riboflavin were determined by the means of the procedure outlined by Conner and Straub (*2*).

SPECTROGRAPHIC METHOD. The vitamin A content of the salmon paste and of the chopped ham was determined by the usual spectrographic method. In the procedure employed, samples of the canned foods were saponified with 5% alcoholic potassium hydroxide, the unsaponified portion was removed by means of a series of extractions with peroxide-free sulfuric ether, the ether being removed under reduced pressure, the residue taken up in a known volume of isopropyl alcohol, and the extinction coefficient measured at 328 millimicrons, using a Beckman quartz spectrophotometer.

In the authors' laboratory each of the six canned foods was assayed for ascorbic acid, carotene or vitamin A, thiamine, riboflavin, pantothenic acid, and niacin, some of the vitamins being assayed by as many as three different methods. Of the 21 laboratories to which samples were supplied, eleven reported assay values. Six of the collaborating laboratories reported ascorbic acid values; 6, carotene values; 8, thiamine values; 7, riboflavin values; 2, pantothenic acid values; and 2, niacin values.

Table II. Vitamin Content of Specially Prepared Canned Foods

(Milligrams per 100 grams of can contents)

Laboratory No.	Method of Assay	Pureed Corn	Pureed Peas	Pureed Carrots	Pureed Tomatoes	Salmon Paste	Chopped Ham
			Riboflavin				
1	Biological	0.119	0.135	0.049	0.088	0.235	0.202
1	Fluorometric	0.103	0.111	0.031	0.065	0.117	0.137
1	Microbiological	0.092	0.090	0.055	0.070	0.230	0.129
2	Fluorometric	0.470	0.400	0.090	0.150	0.200	0.410
2	Microbiological	0.130	0.100	0.060	0.070	0.780	0.250
3	Microbiological	0.076	0.072	0.022	0.043	...	...
4	Fluorometric	...	...	0.024	...	...	...
6	Fluorometric	...	0.089	...	0.052	...	...
7	Fluorometric	0.082	0.159	0.025	0.047	...	...
8	Fluorometric	...	...	...	...	...	0.208
10	Microbiological	0.080	0.087	0.040	0.076	0.190	0.190
			Pantothenic Acid				
1	Biological	0.290	0.200	0.110	0.380	0.510	0.460
1	Microbiological	0.250	0.220	0.160	0.260	0.400	0.640
2	Microbiological	0.200	0.110	0.130	0.120	1.000	0.400
10	Microbiological	0.250	0.140	0.140	0.440	0.630	0.310
			Niacin				
1	Microbiological	1.55	1.56	0.26	1.58	6.70	3.30
2	Microbiological	1.60	1.40	0.18	1.60	5.50	2.70
3	Microbiological	1.55	1.44	0.25	1.38	...	...

DATA AND DISCUSSION

The results of the authors' studies, together with the assay values reported by the collaborating laboratories, are presented in Tables I and II.

ASCORBIC ACID. Examination of the data presented in Table I reveals some rather unexpected variations in the ascorbic acid values as reported by the different collaborators. Pureed tomatoes yielded the most consistent data, while values for pureed carrots showed the widest range of variations. No satisfactory explanation can be advanced for the wide variations in data on the ascorbic acid content of carrots as submitted by the different collaborators. Owing to the fact that several collaborators obtained excellent agreement between vitamin values of different cans of the respective samples, it appears that the initial variation of can contents could, at most, be only a minor contributing factor so far as variability of the assay values obtained. Variations in the storage and the handling of the samples previous to assay and variations in the manipulations during the course of the assay are apparently the major contributing factors.

Since none of the collaborators indicated in his reports that any attempt was made to measure dehydroascorbic acid, no data are available relative to the total ascorbic acid content of the various samples investigated.

CAROTENE. The data on carotene were extremely variable for pureed corn, peas, and carrots, while those for pureed tomatoes were fairly consistent. This is somewhat surprising, since, in the authors' hands, it is much easier to obtain consistent carotene values on corn and carrots than on the highly pigmented tomato products. Here again, it is difficult to attribute these wide variations in carotene values to any single factor or condition. Since no one collaborator reported carotene values which were consistently higher or consistently lower for all four products than values reported by other collaborators, it is doubtful if the variations can be attributed to differences in carotene reference standards, to differences in types of instruments employed in measuring carotene, or to the measurements being made at slightly different wave lengths.

The importance of using standards which have been accurately prepared from highly purified β-carotene cannot be overemphasized. Undoubtedly, many of the irregularities in the carotene data published in the past may be traced to this source of variation. However, with the highly purified β-carotene that is now available and with practically every assay laboratory possessing a precision microbalance, this source of error has been partially eliminated. The use of solutions of impure carotene or the more stable chromate as standards is conducive to considerable error in carotene determinations, especially when these solutions are used under conditions differing from those under which they were standardized. This is due to the fact that the light-absorption curves of these solutions are not identical with that of pure β-carotene. The standard chromate solution offers an excellent means of checking the instrument from time to time but is of very limited value as a carotene standard. In fact, a chromate solution standardized against pure β-carotene in one instrument may give rise to a serious source of error when used as a standard with another instrument owing, primarily, to the differences in the spectral qualities of the lights involved.

In general, carotene values for the four canned products obtained through biological assays, using β-carotene as the reference standard, show relatively good agreement with values obtained by other methods of assay. Carotene values calculated from U.S.P. units (U.S.P. units × 0.0006) were somewhat lower for all four products than when the biological response was based on β-carotene as the standard. This difference is due, in the authors' opinion, to the fact that in their laboratory 0.6 microgram of pure β-carotene gives somewhat less growth than does 1 U.S.P. unit of the reference cod liver oil.

The spectrographic method of assay yielded higher vitamin A values with salmon paste and chopped ham than were obtained by the U.S.P. biological assay. The differences, although appreciable, could in all probability be accounted for on the basis of the interfering materials in the concentrate from these low-potency products. The vitamin A potencies of these products were 210 and 36 U.S.P. units per 100 grams, respectively, by

biological assay and 275 and 63 U.S.P. units, respectively, by the spectrographic method of assay.

THIAMINE. Collaborative reports on thiamine showed greater uniformity than those for the other vitamins. This is probably due to the fact that all collaborators employed the thiochrome method which has been more highly standardized than many other assay procedures.

Thiamine values obtained by the biological method, although somewhat higher in every case, showed relatively good agreement with the collaborative data obtained by means of the thiochrome method of assay, the greater variations occurring among the values obtained for the salmon paste and the chopped ham. Owing to the low thiamine content of the salmon paste, this produce proved extremely difficult to assay biologically, especially by the rat curative method. In order to conduct successful assays of this product by the curative method, it was essential that the test animals be fed a small but known amount of thiamine along with the test material to ensure the ingestion of a sufficient amount of the vitamin to effect a recovery from the paralytic condition and to sustain life for a reasonable number of days. In this instance, the thiamine content of the material being assayed was calculated by difference.

With the rat-growth method of assay, the amount of salmon paste that was required to furnish sufficient thiamine to promote a reasonable rate of growth was great enough to cause a considerable alteration in the ratio of the components of the original basal ration. Thiamine values obtained by the curative and by the rat-growth method of assay showed satisfactory agreement, especially for those canned products containing appreciable quantities of thiamine. For this reason, only the data obtained through the use of the curative technique are reported. The thiamine content of chopped ham, as determined by the biological methods of assay, was considerably higher than that obtained by the thiochrome method. The authors have observed similar findings regarding the thiamine content of other samples of meats assayed in their laboratory. It is probable that the lower thiamine content, as indicated by the thiochrome assay, is due in part to the incomplete extraction of the vitamin from animal tissues.

RIBOFLAVIN. The collaborative riboflavin data showed wide variations (Table II). Here again it is difficult to state which of the methods of assay yielded the most reliable riboflavin values. One collaborator (laboratory 2) reported riboflavin values obtained by the fluorometric method and by the microbiological method of assay in which the values obtained by the former method were considerably higher (150 to 400%) for five of the six products assayed, while the sixth product was found to contain 360% more riboflavin by the latter method. However, riboflavin values obtained by the biological method, although somewhat higher, showed fair agreement with a mean of the values reported by collaborators who employed other methods of assay.

PANTOTHENIC ACID. The results of the collaborative pantothenic acid assays are also shown in Table II. With only three collaborators participating, it is rather difficult to draw conclusions regarding the relative merits of the assay methods employed. In the authors' hands, the rat-growth method and the microbiological method yielded results which show essentially the same correlation that exists between data obtained by the three collaborating laboratories through the use of the microbiological technique.

NIACIN. Niacin values reported for the four vegetable products by three collaborators employing the microbiological method of assay show excellent agreement.

The data reported are instructive in that they indicate to some extent the variations in the apparent vitamin content due to different methods of assay and, to a greater extent, the variations which are attributable to the foods being assayed under somewhat different conditions and by different technicians. Such results do not reflect particularly upon the efforts of the few collaborators who participated in these studies. It is highly probable that the assay data reported may show a higher degree of correlation, in general, than would the data obtained by those would-be collaborators who requested corresponding samples but failed to report the results of their assays. It is believed that many of the data reported in the literature relative to the vitamin content of various foods are subject to even greater errors than the errors indicated in this report. It also seems highly probable that many of the variations in vitamin content which have been attributed to varietal differences, seasonal variations, stage of maturity, climatic conditions, storage conditions, processing methods, etc., are due in part to inaccurate assays. This is especially true where attempts have been made to compare data reported by different laboratories.

SUMMARY

Six representative foods (corn, peas, carrots, tomatoes, salmon, and ham) were prepared as purees or pastes to ensure uniformity of composition, processed by commercial methods, and packed in tin containers. The resulting products were assayed for ascorbic acid, carotene (or vitamin A), thiamine, riboflavin, pantothenic acid, and niacin by several collaborators, using some of the accepted methods of vitamin assay.

The data obtained indicate clearly the need for more extensive collaborative studies regarding some of the methods of vitamin assay, especially from the standpoint of the application of these methods to the determination of the vitamin content of a wide range of foods. There are undoubtedly many factors which have contributed to the variability of the data reported. From the available information, however, it is difficult to state which of these factors has contributed most toward these variations. The use of different vitamin standards by different collaborators does not appear to have been the major contributing factor so far as the variability of the data is concerned. Likewise, the use by different collaborators of different types of physical equipment during the course of the respective assays does not appear to be a major source of error. It appears that the human element is, perhaps, one of the most significant sources of variable results, although the data presented do not indicate the magnitude of variations arising from this factor.

The fact remains, however, that existing methods of vitamin assay do not always yield concordant data in the hands of different investigators when applied to the same food products. This is a matter which must be corrected before the relative merits of vitamin data reported by different laboratories can be evaluated accurately. Variations in assay values due to personal error must be eliminated through extensive training of the technician and through subsequent experience with the method of assay. Furthermore, methods of vitamin assay which yield satisfactory results with one food often yield unsatisfactory results when applied to another food. This is due to the fact that foods differ widely, in both their chemical and physical characteristics. Modifications of assay techniques to adapt them for use with a wide range of products will be revealed only through extensive applications of the techniques to each of these products. This type of information can be most readily obtained through collaborative studies. To yield results of maximum scientific value, a collaborative study of this type should ensure (*a*) that representative samples of unquestionable uniformity be prepared in quantity from the same initial source, (*b*) that these samples be stored under the same conditions prior to assay, (*c*) that stable vitamin reference standards be placed at the disposal of each collaborator, and (*d*) that each collaborator use the same methods in which all details of procedure and quality of reagents are carefully prescribed.

EFFECT OF MINOR MODIFICATIONS IN TECHNIQUE. So far as the authors are aware, there is no way of accurately ascertaining the probable consequences of certain minor modifications of assay techniques on the vitamin values obtained. Even if com-

plete details were available as to the deviations from the recommended assay procedure, as practiced in some collaborating laboratories, it would be difficult to conclude from such data that any one deviation was solely responsible for the inconsistencies in assay values. It is realized that all vitamin assay laboratories are not similarly equipped, that they do not all have the same intensity of illumination, that different grades of reagents and solvents are frequently used in different laboratories, that different types and makes of instruments are used in measuring vitamin concentration, and that laboratories frequently differ in the facilities available for storing or holding vitamin-containing foods, extracts, solutions, etc. The authors believe that variations of this type may be reflected in the final results of the assay and that the inconsistencies in the data reported are not always the fault of the method. Investigators who do not have the facilities recommended by the author of the method frequently proceed to use available facilities and materials without fully realizing that they are not complying with the requirements of the method.

There have been proposed certain changes or modifications of some of the assay methods employed in these studies which are supposed either to increase the accuracy of the methods or increase their application to a wider range of food products. In some instances these modifications have tended to simplify the method and at the same time increase its usefulness. There are, however, investigators who insist that at least some of the suggested revisions offer no significant advantage over the original method. The fact that the methods cited in this manuscript continue to be used rather extensively in their original form is proof in itself that the proposed modifications are not too generally accepted.

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PUNCH CARDS IN CORRELATION STUDIES

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THE recent article by Cox, Bailey, and Casey (*1*) has called attention to the usefulness of punch cards in chemical bibliography. Experience gained in two years of work in this laboratory has led to the conclusion that punch cards are adaptable to a wide variety of chemical problems, particularly those which deal with large groups of data, such as are encountered in surveys, correlations, or extended experimental studies.

The particular problem we had here was a statistical investigation of the correlation between chemical structure and toxicity towards insects and fungi. From a search of the literature and other sources (*2*), data were collected on approximately 8,000 compounds, on each of which one or more toxicity tests had been made. With such a large number of compounds, and such a wide variety of constituent groups, counting and correlating the data by inspection promised to be a formidable task.

The first step in the use of the punch cards was the preparation of a list of all of the constituent groups present in the compounds under study. These were arranged in order of decreasing complexity, and to each was assigned a number. The chemical formulas for each of the 8,000 compounds were then broken down into constituent groups as they appeared on the master list, and the numbers representing these groups recorded for each compound. For example, opposite ethyl alcohol the numbers 581 and 1011, designating hydroxyl and ethyl groups, were recorded.

To supply the needed information, it was necessary to prepare as many punch cards for each compound as there were constituent groups in that compound. Thus, for the example already given, two cards were prepared, one of which was punched with the number 581, the other with 1011. These numbers were punched on the lower margin of the cards, using the 7-4-2-1 "field" arrangement described by Cox *et al.* On the right margin of the card was punched information on the broad class of compound (insecticide, fungicide, organic, inorganic, etc.), while on the left margin was punched a number representing the relative toxicity of the compound, based on the number of tests, and their results. An index or acquisition number was given each compound and was punched on the top margin of the card. Information of a general nature, including the name and formula of the compound, all constituent numbers, and literature reference numbers were typed on the face of each card.

After the cards were prepared, the individual constituent groups were separated by sorting. To return to the example, by sorting for number 581, all compounds having a hydroxyl group in the molecule were collected together. The results of the toxicity tests were then totaled, the sum being a numerical designation of the toxicity of compounds containing this particular group. As an extension of this idea, it is relatively easy to study the effect of combinations of constituent groups when present in the same molecule.

By slight modifications of these basic principles, it is possible to make correlation studies between chemical constitution and any desired property, chemical or physical. With the multiplicity of information accumulated in the literature, it would appear that the punch card will be a most useful tool in chemical research.

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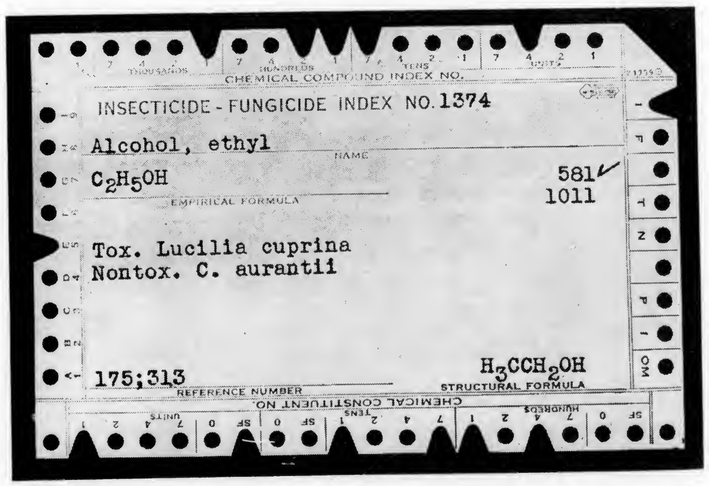

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Problems Concerning Frozen Foods*[1]

By
John E. Nicholas[2] and N. B. Guerrant[3]

THE preservation of foods by freezing is receiving wide public acceptance and the practice is rapidly approaching the magnitude of a major industry. In view of the rapid growth of the industry and of the diversified opinions relative to the most satisfactory procedure to be followed in selecting, preparing and freezing various foods, it is to be expected that many of the present practices are not standardized either with respect to organization, equipment, or operation.

In this article the authors discuss the problems involved in selecting, preparing, packaging and processing which affect the quality and nutritive value of frozen foods. Because of the ever-increasing popularity of food freezing, the authors believe that a sound evaluation of freezing techniques should be the goal of future research efforts.

Questions of Interest on Frozen Foods

A REVIEW of the literature relating to frozen foods indicates to the authors that considerable disagreement exists in the minds of various investigators as to certain fundamental practices pertinent to the successful freezing of foods. Among the questions of particular interest are: (1) At what stage of maturity or ripeness should the product be harvested in order that it may produce the most wholesome food? (2) What is the best procedure to be followed in the preparation of a specific food for freezing? (3) What is the most desirable type, shape and size of packaging for use with a specific product? (4) How quickly must the food be frozen in order to preserve its maximum nutritive value? (5) When once frozen, at what temperature must the food be stored in order to yield the most wholesome product? (6) What is the most satisfactory procedure to be followed in preparing the frozen food for human consumption? (7) What are the most tangible and reliable characteristics of quality in frozen foods?

From the available information, it seems obvious that the answers to these questions are not simple and that urgent need exists for a more fundamental and extensive research. Especially is this true when consideration is given to the vast differences in the nature and in the composition of the various foods which may be preserved in the frozen state with varying degrees of success[22]. Due to the problems involved, the questions are briefly discussed in the order listed.

Stage of Maturity or Ripeness

WHILE much remains to be learned regarding the influence of stage of maturity or ripeness of various fruits and vegetables on the subsequent quality of the frozen products, sufficient information is available to indicate that stage of maturity or ripeness is of greater concern where the food is to be frozen than if it is to be preserved by canning or by dehydration. The importance of varietal differences should not be underestimated. It appears, however, that at least some of the differences commonly attributed as being of varietal origin are primarily matters of maturity or degree of ripeness. Fitzgerald[13] concluded that the state of maturity had perhaps the most important single influence upon vitamin C content of any vegetable used for freezing. Mack, Tressler, and King[17] have reported on the deleterious effect of maturity on the vitamin C content of several varieties of peas. In general, over-mature and fibrous vegetables do not lend themselves to successful freezing. Likewise, unripened, over-ripened or unsound fruits do not become wholesome products when preserved in the frozen state. To insure success in the preservation of fruits and vegetables by freezing, only products of the highest quality should be used. Since different food products and different varieties of the same products may attain optimum quality at different stages of maturity, the importance of stage of maturity merits further study.

Factors in Preparation

IN the preparation of food for freezing, far too little consideration has been given to physical and chemical changes which take place between the time the products are taken from the tree or the vine and when freezing actually is accomplished. Both fruits and vegetables should be prepared and placed in the freezer as soon as possible after harvesting. All such products intended for freezing must undergo the usual sorting, picking, pitting, paring, etc. It seems preferable that this be done in the cool or early part of the day. Boggs, Campbell, and Schwartze[5] in their studies on factors influencing the texture of peas preserved by freezing, report that vining toughened both the skins and cotyledons of

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frozen peas; however, a delay between vining and freezing toughened the skins, but not the cotyledons, with most of the toughening occurring in the first four to six hours of delay. Since many fruits and vegetables deteriorate rapidly in quality when stored at room temperature for any extensive period, those products which cannot be processed immediately should either be packed in crushed ice or stored at a temperature only slightly above freezing. One of the common treatments to which most vegetables are subjected before freezing is scalding. The main purpose in scalding is to inactivate the enzymes which abound in fresh plant tissues and which result in the deterioration of the frozen product, especially during prolonged periods of storage. Campbell[5] in his studies with corn found evidence that the quality of frozen cut corn may be markedly improved by scalding on the cob prior to cutting and keeping the cut corn out of contact with water. According to this investigator the adequacy of the scald obtained under each operating condition of volume, time and temperature should be carefully checked with all products.

Two types of scalding are currently used, hot water and steam. Up to the present time steam scalding has experienced rather limited usage, owing partly to the relatively high cost of the necessary equipment and partly to the fact that this type of scalding has not thus far proved applicable in the processing of some leafy vegetables. Hot water scalding, on the other hand, is receiving a very wide usage, although it appears quite certain that this treatment results in the extraction of a greater amount of the water soluble nutrients from the scalded product than does steam scalding. The length of the blanching period may influence the vitamin content of quick frozen vegetables. Because of the solubility of these vitamins in water, Fellows *et al.*[11] recommend a minimum blanching period consistent with the requirements of the particular product. These investigators have used 50 seconds for peas; 1.75 minutes for spinach; 2.5 minutes for asparagus; and 3 minutes for lima beans. It is needless to state that ideal conditions for scalding vegetables seldom if ever are encountered; no phase of frozen food processing merits more thorough scientific investigation than that pertaining to the proper preparation of vegetables for freezing.

Fruits, after being prepared by paring, coring, or slicing usually are sweetened either with granulated sugar or with a thick sugary syrup, placed in moisture-proof containers and frozen without further treatment. The sugar or syrup serves not only as a preservative of food value but also tends to preserve natural color by providing an environmental condition unfavorable to oxidation.

With most meats preparation for freezing is rather a simple matter. The product usually is incased or wrapped in a moisture and odor-proof membrane or paper and then placed directly in the freezer.

Containers

THE prime function of the package in frozen food storage is (1) to retain the shape of the original pack, particularly fruits or vegetables, (2) to prevent desiccation and (3) to protect foods from absorbing flavors. These requirements tend to keep the frozen product in a wholesome, presentable and sanitary condition. The wrapping or packaging material should be substantial to prevent tearing if products are handled frequently during storage. According to Birdseye[2] one of the most difficult problems to solve in connection with the rapid growth of the frozen food industry has been, and probably will continue to be, the development of the best type or types of containers for the frozen product. Metals, rubber, synthetics, and ice coating will undoubtedly play important parts and will provide interesting phases in the development of packaging methods. Rabak[23] reports that the extent of weight loss in storage of frozen foods is governed by the relative moisture-vapor proofness of the lining or wrapping materials and its thoroughness of sealing. Frozen foods which undergo excessive weight loss (5 per cent or more) in storage, lose color and have other physical properties impaired. However, the degree of destruction of ascorbic acid was found by this author not to be influenced by desiccation during storage, but by storage temperature.

According to Cook[6] there exists an urgent demand for the development of a package capable of being easily and effectively sealed. The full benefit of a relatively impermeable liner can be contained only if it is completely sealed to prevent surface drying.

Rate of Freezing

MUCH disagreement prevails regarding how quickly foods must be frozen to retain maximum food values. If foods are packaged or wrapped before freezing, experimental evidence shows that an exceedingly large heat transfer barrier is introduced, thus increasing the time required to freeze the product. It is not only the size of the package that prevents rapid freezing but also the number of layers of wrapping material. All other factors being equal, the greater the surface exposed, the faster the product will freeze.

Since the preponderance of evidence indicates that rapid freezing is an important consideration from the standpoint of quality of final product, and since packaging or wrapping materials have been found to retard the rate of freezing materially[21] it appears that freezing the products, then packaging, is highly desirable, with certain foods, and may be a more general practice in the future.

Opinions vary relative to the influence of freezing processes on cell structure and on resulting product. According to Diehl *et al.*,[8] the physics and chemistry of plant tissue freezing are not the same as those which characterize the freezing of animal tissue. These authors have shown experimentally that in the freezing of tissues ice crystallization begins in the intercellular spaces and not within the cells themselves, as formerly believed. Thus, the ice crystals grow in size through crystallization of water drawn from the cell. These investigators also observed that this process may continue until cell shapes are pressed and distorted by masses of ice which in rapid freezing at very low temperatures may rupture the cell walls. While the physiological character of the cell contents may be altered, the piercing of cell walls by ice crystals and mass-crushing of protoplasmic materials probably play a less important role than has been supposed. According to Joslyn and Marsh[15] the investigation of the temperature changes during freezing of fruits and vegetables is of major importance to the industry and should be done before definite recommendations are made for the packing of foods for freezing.

Woodroof[28] reports that in instant freezing, where temperatures from —80 to —100°F. were used, a product was produced which was superior to that produced by all other systems of freezing. The preservation of plant and animal tissues by subjecting them to very low temperatures is a complicated phenomenon. Although the physical effects of very low temperatures on plant tissue have received considerable attention, more fundamental research is needed. Birdseye[2] believes that rapid freezing is extremely important in the forming of ice crystals sufficiently small so as not to rupture cell walls. Others contend that preparation for freezing is more important than speed of freezing. In support of the latter view, Woodroof[29] obtained more satisfactory results with figs frozen at zero temperatures or higher than at lower temperatures. Due to the spongy nature of starch products, such as peas, corn, and lima beans, which have the capacity to release and reabsorb quantities of water without altering their structure, this investigator found[31] that rapid freezing was not as important for these products as it was for spinach and asparagus.

Storage Temperatures

IT has been held axiomatic that the lower the temperature at which foods are stored the longer the safe storage period. An important economic consideration is, however, whether —10°F. provides a storage environment comparable to that at —30°F. While low temperatures undoubtedly increase costs of storing foods, it is no less important to determine the effects of temperature variations on individual products during storage. Such variations may prove to have marked influences and in varying degree on the different products.

That temperature of freezing and length of storage have some significance on beef have been observed by Ramsbottom and Koonz[24] who state that the amount of drip, after defrosting, was affected both by temperature and time of storage. These investigators also observed little difference in the ice crystal structure of frozen beef stored at 10° and —30°F., neither was there a significant change in the pH of beef stored for one year at these temperatures. But a greater oxidation and consequently darker beef occurred in the superficial tissues stored at 10°F. than occurred at —30°F.

Brady *et al.*[4] found that slow frozen meat has a higher evaporation rate during storage than quick frozen meat. In their studies the smallest cooking loss (drip and evaporation) occurred in quick frozen steaks which were broiled while still frozen and the largest loss occured in slow frozen steaks, thawed before broiling. These investigators believe that cuts of meats should be quick frozen and cooked while still frozen to prevent a high loss in evaporation and drip, even though there is no apparent effect on the palatibility of steaks prepared this way.

Experimental evidence (Woodroof[30]) has shown the tissues of fruits and vegetables after complete defrosting to be flabby and to have lost from 20 to 80 per cent of their weight by leakage in 24 hr. This loss was not due to rupture of cell walls but to the irreversible osmosis of cell contents. A most significant observation was that freezing at different temperatures produces slightly different effects on the cell contents, these differences being of degree and not of kind. Regardless of storage temperature used, ice crystals do not break down the cell walls but cell contents instead, and produce not only death of cells but collapse of cellular structure. Evidence indicates that each product has a rather definite optimum temperature at which it should be frozen and stored; a temperature above or below produces a less satisfactory product.

Preparing Frozen Foods for the Table

THERE remains much to be learned regarding the most satisfactory methods of preparing frozen foods for human consumption. However, it has been well established that frozen foods, in general, deteriorate much more rapidly after thawing at room temperature than do the corresponding fresh unfrozen foods. Likewise, it has been established that foods which have been frozen lose to their cooking water and their juices larger amounts of soluble nutrients than do similar foods which have not been frozen. From these facts, it appears that frozen foods must be cooked immediately after thawing if the maximum quality is to be retained. In many instances it has been found desirable to place the frozen product directly into the cooking utensil and apply heat while the food is still in the frozen state. Frozen foods should be cooked in a minimum amount of water. This is especially true if the cooking liquids are to be discarded. Since most frozen fruits are eaten immediately after thawing without subsequent preparation, they do not appear to be subject to those special conditions of preparation that frozen meats and frozen vegetables require. McFarlane[18], reporting on cider frozen and stored at 14° and —4°F., found viable microorganisms decreased most rapidly in sections high in soluble solid content at —4°F. In samples frozen at 14°F. the greater number of viable cells, after 31 weeks, were found in the unfrozen concentrate which had approximately six times greater soluble solid and total acid content than normal cider. During the latter part of a 26-week storage period viable microbial counts in raspberries frozen at —4° were slightly greater in sections from the lower halves of containers which were a little higher in soluble solid content. In peas frozen at —4°F. for 24 weeks, the viable microbial distribution tended to be concentrated in a central cone-shaped area and in a surface area; but large numbers of microbial forms remained viable for 24 weeks at both temperatures in brine packed peas.

More knowledge is required regarding microbial decomposition of frozen foods, particularly of the types and species of organisms involved, of their prevalence, and of their physiological behavior in the environmental conditions prevailing during the preparation, freezing, storage, and ultimate thawing.

Quality in Frozen Foods

PERHAPS no one phase of the frozen food problem has produced so much confusion in the minds of the food technologist as has the matter of quality. Up to the present time the term "quality" when applied to frozen foods has been indiscriminately used. This has been particularly true in the case of those investigators who do not comprehend the true complexity of the many factors involved. To some the term quality appears to apply to that intangible something which they are unable to describe. To others, it appears to be merely a synonym for the word taste. And still to others, it seems to be masked in that all-inclusive word palatability. Unfortunately, none of the above criteria of quality to date have been measured in terms of a concrete standard and, in consequence, quality becomes a matter of personal opinion. Since personal opinions are both variable and fallible confusion prevails. Three criteria, by which the quality of most frozen vegetables can be accurately determined, according to

Tressler,[27] are bacterial count, enzyme, and ascorbic acid content.

It is highly desirable that the characteristics of quality be defined in terms of measurable factors. When this has been done, the basis has been established for collaborative research in the field of frozen foods. It seems logical to suppose that if and when such factors have been established they will include among other things, color, texture, enzyme action, and bacterial count, as well as nutritive value with special reference to certain vitamins, and probably taste and odor.

Future Outlook

The various procedures involved in the preservation of the frozen foods of today are, for the most part, the results of a trial and error approach. A few of the more interested industrial companies, as well as a limited number of institutional research laboratories, have attacked some of the more troublesome problems concerned from a scientific viewpoint. In a consideration of the numerous and complicated factors involved in quality studies, Geer[14] believes that the technic of sampling is of utmost importance. The frozen food industry is still in its infancy and, therefore, many of the practices in use today may be found to be wholly obsolete in the next few years. No mode of food preservation offers a greater challenge to the scientifically trained researcher. Food technologists, engineers, and other technically trained individuals, working in the frozen food field, should pool their ideas and data so as to form the basis of a more fundamental approach in the realm of the frozen food technology. For example, there appears to be a great deal of misunderstanding as to what the terms "quick freezing" and "slow freezing" actually mean.

Investigators agree[30] that as the frozen food industry develops, a demand will be made for a more refined technique in judging food quality as it is affected by processing, by rate of freezing, by temperatures during freezing and storage, by variations in packaging, and by what may be termed a time-temperature factor in storage. A sound evaluation of freezing techniques with respect to their use in the preservation of maximum nutritive values and other qualitative characteristics of frozen foods should become a common goal of future research efforts.

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Nutritive Value of Canned Foods

INFLUENCE OF TEMPERATURE AND TIME OF STORAGE ON VITAMIN CONTENTS[1]

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The effect of storage on the vitamin content of tomato juice, green lima beans, and whole-kernel yellow corn has been studied at 30° to 110° F. over a 365-day period. The vitamin content of each food is affected adversely by prolonged storage at the higher temperatures. The amount of vitamins retained varies with the type of product and the particular vitamin. Both time and temperature affect the retention of ascorbic acid. When corn and lima beans are stored at higher temperatures for long periods, spurious reducing substances are formed which interfere with ascorbic acid determination. Temperature of storage has a limited effect on carotene retention but a marked effect on thiamine retention. Riboflavin retention is not seriously affected by the conditions employed. Pantothenic acid retention is affected adversely by both time and temperature of storage, the temperature effect being most significant. Storage at extremely high temperatures (110° F.) for relatively short periods results in marked losses of vitamins, especially ascorbic acid and thiamine. These data emphasize the necessity of storing canned foods under the most favorable conditions in order to retain maximum vitamin content. It is suggested that the vitamin content of canned foods is preserved most effectively at storage temperatures ranging from just above the freezing point of the respective food to approximately 42° F.

NUMEROUS investigators have reported on the nutritive value of canned foods, but relatively little information is available regarding the previous history of the canned products described. It is reasonable to conclude (*1*, *2*, *6*, *11*, *15*) that many conditions are operating which affect the nutritive value of the finished product; among them are such factors as variety, growing conditions, stage of maturity at time of harvest, methods of handling between harvesting and canning, and methods of blanching and processing.

Information is also lacking on the changes in nutritive value which are likely to occur from the time commercially canned foods are processed until they reach the consumer. Time and temperature of storage are factors which may cause significant changes in the nutritive value of canned foods. This is true, particularly, with reference to changes in vitamin content.

Daniel and Rutherford (*4*) showed that home-canned tomato juice, stored at room temperature for 6 months, sustains an appreciable loss of ascorbic acid. Roberts (*12*) reported that grapefruit juice, stored for 9–15 months, lost about 25% of the ascorbic acid. The temperature of storage was not specified. Fellers and Buck (*5*) reported that strained peas, spinach, and tomato juice, packed in glass and stored for one year at room temperature, lost from 10 to 25% of the original ascorbic acid and that little loss of this vitamin occurs after the first 2 or 3 months of storage. Glass-packed foods stored for one year at 36° F. lost but 5% of the ascorbic acid; similar products stored at 90° F. for a similar period lost approximately 21%. These investigators report that glass-packed strained spinach, carrots, peas, and tomato juice retained approximately 85% of the original carotene after storage for one year in subdued light. While the temperature of the storage was not given, the authors conclude that "there is a small but significant loss of vitamin A in canned peas, spinach, and tomato juice when held under rather adverse storage conditions".

Moore and co-workers (*7*) made a study of the changes occurring in orange and grapefruit juices during commercial processing and subsequent storage. Both products were stored in glass and in tin containers at 40° and at 80° F., respectively, for 6 months. Ascorbic acid assays were made at monthly intervals. Their data show that ascorbic acid retention was greater in the canned than in the bottled product, and that the retention of this

[1] This is the thirteenth of a series of papers dealing with the general subject. Previous articles have appeared in *The Journal of Nutrition*, *Journal of the American Dietetic Association*, and ANALYTICAL EDITION OF INDUSTRIAL AND ENGINEERING CHEMISTRY (August issue, page 531; November issue, page 710).

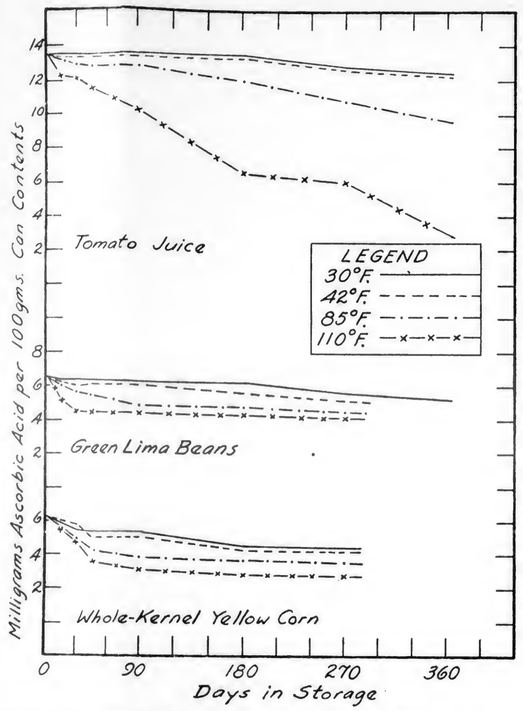

Figure 1. Effect of Time and Temperature of Storage on Ascorbic Acid Content of Canned Tomato Juice, Lima Beans, and Yellow Corn

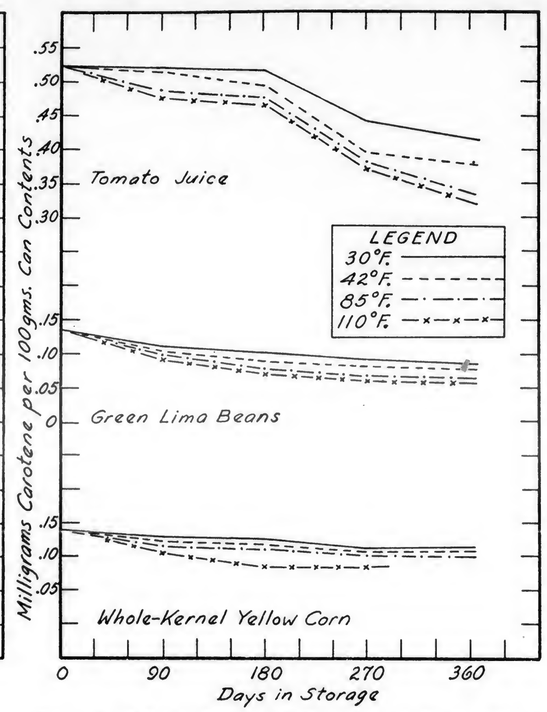

Figure 2. Effect of Time and Temperature of Storage on Carotene Content of Canned Tomato Juice, Lima Beans, and Yellow Corn

nutrient was considerably greater at the lower storage temperature (40°) than at the higher (80° F.).

It seems evident that both time and temperature of canned food storage play a role in determining the vitamin content of the retail product. Temperature of storage appears to have a more significant effect on the retention of ascorbic acid than time of storage; the reverse appears to be true with carotene. Little or nothing is known about the effect of these factors on the retention of some of the other vitamins. The influence of storage temperatures in excess of 80° F. and of storage periods lasting more than 6 months on the vitamin content of canned foods have not been sufficiently investigated.

The armed forces were frequently confronted with the problem of storing vast quantities of canned foods for extended periods and sometimes under adverse conditions as far as temperature was concerned. The present report covers some of the findings obtained in a preliminary investigation of this problem.

STORAGE CONDITIONS

Three canned food products were used in these studies. Each of the three represented a commercial pack.

Tomato Juice was prepared and canned in No. 300 cans on August 20 from tomatoes (Rutgers) grown on sandy loam soil in the state of Delaware. Because of the comparatively wet growing season, the tomatoes arrived at the cannery in what was considered "fair" condition. They were processed within 10 hours of the harvesting time. Before processing, the tomatoes were washed in an Ayars washer, then spray-rinsed in a reel washer. The juice was processed for 20 minutes at 212° F. in a three-crate vertical retort and was then canal-cooled. The finished product graded "fancy" in accordance with the canner's standard.

Whole Kernel Yellow Corn (Golden Cross), grown in Maryland during a rather wet season, was prepared and canned on August 23. The corn was processed within 6 hours after harvesting. The product was washed in a combination flotation and reel washer. The corn was brine-packed in No. 2 cans, processed in a vertical retort for 50 minutes at 240° F., and then canal-cooled. According to the canner's standard, the product graded "fancy".

Green Lima Beans (Henderson's bush limas) grown on sandy loam soil in the state of Maryland, were prepared and canned on August 22. The product arrived at the cannery in average condition due in part to the unusually damp season. The beans were canned within 10 hours after harvesting. After being shelled, the beans were dry-cleaned, washed in a universal washer, and subjected to quality separation. The storage studies reported concern only that portion of the batch which came from the quality separator under the classification "medium greens". The beans were water-blanched for 2.5 minutes at 190° F., brine-packed, and processed in a vertical retort for 20 minutes at 240° F. The processed product was canal-cooled. In accordance with the canner's grading system, the finished product graded "fancy".

More uniformity of product was achieved by selecting cans of foods which were being processed in sequence from the same batch of canning stock. The canned foods were delivered at the laboratory as soon as possible after processing; there the cans were redistributed among the various cases so as to effect a uniform allocation of the canned product under the various conditions of storage. After representative cans were selected for the initial vitamin assays, the foods were stored at 30°, 42°, 85°, and at 110° F. The highest temperature (110° F.) was selected with extreme conditions of Army storage in mind; it is considerably above what would be expected domestically. At specified intervals, representative cans were removed from storage; the contents were assayed for ascorbic acid, carotene, thiamine, riboflavin, and pantothenic acid, and also examined as to quality and solid-liquid distribution. In addition, each can was examined as to vacuum and condition.

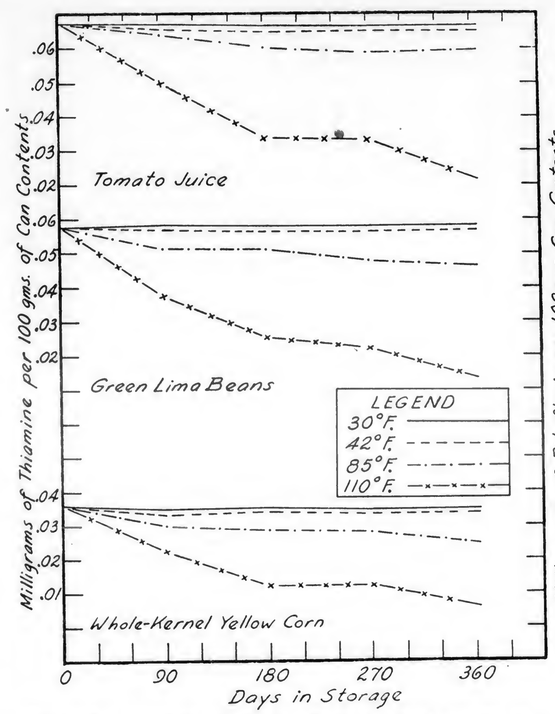

Figure 3. Effect of Time and Temperature of Storage on Thiamine Content of Canned Tomato Juice, Lima Beans, and Yellow Corn

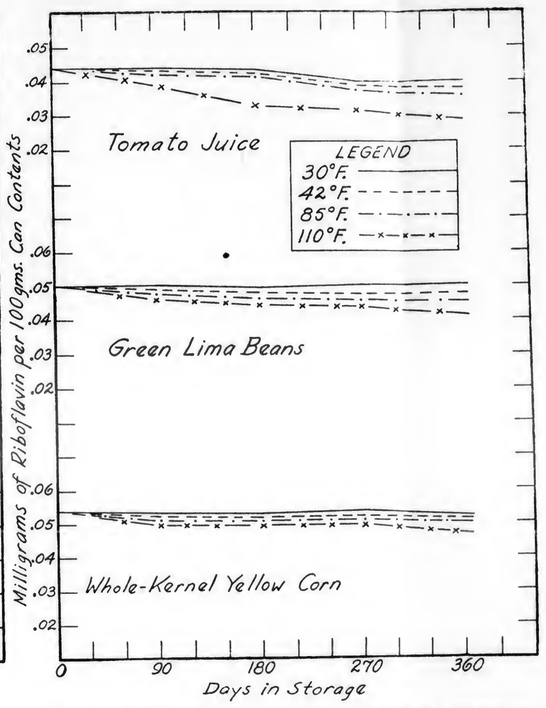

Figure 4. Effect of Time and Temperature of Storage on Riboflavin Content of Canned Tomato Juice, Lima Beans, and Yellow Corn

In most instances assays were made on aliquots from a well-mixed composite consisting of the contents of six cans. With the exception of those used in the ascorbic acid determinations, all aliquots were reduced to a uniform consistency by blending in a Waring Blendor before the samples were weighed. Aliquots used in the ascorbic acid determination were mixed with a known amount of metaphosphoric acid solution of definite concentration before blending.

Each of the three products was assayed for the above-mentioned vitamins when placed in storage. Additional ascorbic acid assays were made after 15, 30, 45, 90, 180, 270, and 365 days of storage. Assays for the other vitamins were made after 90, 180, 270, and 365 days of storage.

Ascorbic acid was determined by the procedure essentially as outlined by Morell (*9*); metaphosphoric acid was employed as the preservative and an Evelyn photoelectric colorimeter as the measuring instrument. Carotene was determined by the method outlined by Moore and Ely (*8*), petroleum ether (boiling range 35–60° F.) being used as the solvent. In the early part of the investigation carotene measurements were made with a Cenco-Sheard spectrophotelometer, but during later stages a Beckman quartz spectrophotometer was employed. All carotene measurements were made at a wave length of 450 microns.

Thiamine and riboflavin were determined by the method of Conner and Straub (*3*). In each instance the fluorescence was measured in a Model 12, Coleman electronic photofluorometer. Riboflavin was also determined by the microbiological procedure of Snell and Strong (*14*). Pantothenic acid was determined by the method of Pennington, Snell, and Williams (*10*).

VITAMIN CONTENTS

The data are presented in condensed form in Figures 1 to 5. They indicate that the vitamin content of each canned food was affected adversely by storage at the higher temperatures for relatively short periods. The amount of the original vitamin retained by the three products varied somewhat with the food under investigation and also with the different vitamins. In general, the effect of time of storage on vitamin retention was less marked than that of temperature.

Tomato juice retained 92% of the original ascorbic acid after storage for one year at 30° F.; only 20% of this vitamin was present after the product had been stored for the same period at 110° F. (Figure 1). Green lima beans retained 83% of the original ascorbic acid when stored for 270 days at 30° F., and 65% appeared to be present after storage at 110° for the same period. Yellow corn showed favorable retention of ascorbic acid (92%) when stored at 30° F. for 270 days but rather poor retention when stored at 110° (47%).

In general, storage at 42° F. for 270 days resulted in a favorable retention of ascorbic acid by all three products. When stored for the same period at 85° F., the loss in ascorbic acid appeared to be approximately half of that occurring while the products were maintained at 110° F. For some unknown reason the apparent ascorbic acid content of the yellow corn and of the lima beans not only ceased to decrease throughout the course of the storage period but actually appeared to increase as a result of prolonged

storage (9 to 12 months) at the higher temperatures. These apparently anomalous ascorbic acid values appeared to be due to the formation of nonascorbic acid compounds which reacted with the dye during the course of the assay and therefore indicated abnormally high ascorbic acid contents. Attempts to check the reliability of the ascorbic acid values through additional analyses by the method of Roe and Oesterling (*15*) did not reveal either the nature of the interfering substances or their probable concentration in the various samples.

When the indicated ascorbic acid values for both corn and lima beans were greater after 365 days of storage at the higher temperatures than they were after a 270-day storage period, the former values were considered unreliable and, therefore, are not indicated on the graph. It is impossible to state at what stage of the storage period this spurious reduction began to vitiate the true values; therefore the data obtained for the ascorbic acid content of corn and lime beans after prolonged storage especially at the higher storage temperatures, are of questionable accuracy.

With all three products carotene retention appeared to be less affected by temperature of storage than was ascorbic acid (Figure 2). Each of the products, regardless of storage temperature, showed measurable losses of carotene. In general, the losses increased with increase in storage temperature. Tomato juice, lima beans, and yellow corn retained 79, 78, and 74%, respectively, of the original carotene when stored at 30° F. for one year. Tomato juice and lima beans retained only 62 and 54%, respectively, of the original carotene content when stored for a similar period at 110°. Yellow corn, for some unknown reason, reacted peculiarly to prolonged storage at the higher temperatures in that its carotene content decreased rather consistently with respect to both time and temperature of storage throughout the first 180 days of storage. At this date, corn stored at 30° F. retained approximately 90% of the original carotene, whereas corn stored at 110° retained only 58%. With continued storage, however, yellow corn held at the higher temperatures appeared to increase slightly in carotene content; the same product, maintained at the lower temperatures (30° and 42°), continued to decrease in carotene content throughout the remainder of the 365-day period. Further investigation is being carried out with the hope that an explanation may be found for this anomaly.

Of the several vitamins studied, thiamine retention proved to be least affected by time of storage but one of the most severely influenced by temperature of storage (Figure 3). At the end of a 365-day period at 30° F., all three foods retained essentially 100% of their original thiamine content. Thiamine retention at the 42° storage was also favorable. However, when the three products (tomato juice, lima beans, and yellow corn) were stored at 110° for one year, only 31, 25, and 20%, respectively, of the original thiamine was retained. Measurable losses of thiamine were also detected as the result of storage at 85° F.

Retention of riboflavin by each of the three products, although affected by both time and temperature of storage, remained relatively satisfactory during the entire period. Both fluorometric and microbiological methods of assay were used in determining the riboflavin content of these foods; however, only those data obtained by the microbiological method are given (Figure 4), owing to similarity of values and of trends in riboflavin retention as indicated by the results obtained by the two methods. For the tomato juice the two methods of assay yielded almost identical results for all periods. With the lima beans and the yellow corn, the fluorometric method yielded slightly but consistently higher riboflavin values. The data show that, under the most unfavorable storage condition employed in this investigation, approximately 60% of the original riboflavin content was retained after 365 days. Of the three products studied, the riboflavin content of yellow corn was least adversely affected by storage; that of tomato juice was most severely affected.

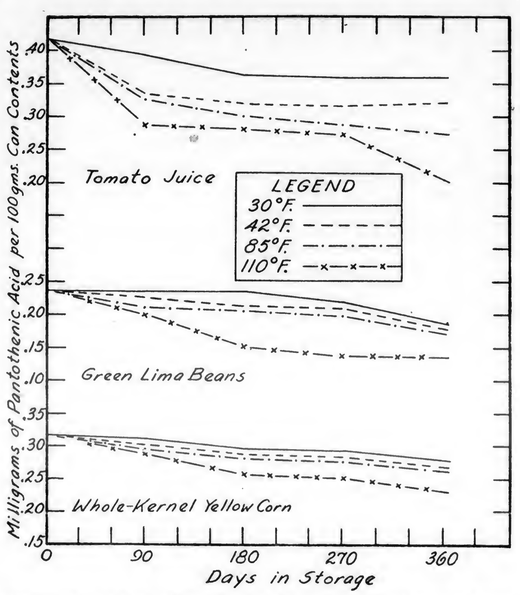

Figure 5. Effect of Time and Temperature of Storage on Pantothenic Acid Content of Canned Tomato Juice, Lima Beans, and Yellow Corn

The pantothenic acid content (Figure 5) of the three foods was not seriously affected by time of storage, but the temperature effect was somewhat significant in the case of lima beans and yellow corn and definitely significant for tomato juice. When stored for one year at 30° F., tomato juice retained approximately 85% of its pantothenic acid content while 45% of the original pantothenic acid remained after storage at 110° F. for the same period.

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The Application of Liquid-Liquid Extraction to the Fractionation of Coconut Oil [1, 2]

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BECAUSE of the complex composition of fats and oils and the difficulties which their analysis presents, comparatively little is known with respect to their glyceride structure. The methods available to chemists for investigations of the component glyceride composition of fats and oils have been limited. Prior to 1927 fractional crystallization was the only method available; by means of this technic small yields of certain individual higher-melting simple and mixed triglycerides were obtained from various sources. A discussion of this earlier work will be found in the review of Hilditch (1). In recent years interest in fractional crystallization as a method of fractionating glycerides has been revived (2). In 1927 Hilditch and Lea (3) introduced the permanganate oxidation method, by means of which Hilditch and his collaborators have been able to investigate quantitatively the glycerides of fats and oils in terms of their saturation and unsaturation (1). During the past several years, molecular distillation has been applied to the fractionation of glyceride mixtures, but the results have not been encouraging as far as the elucidation of glyceride structure is concerned. The literature in this field was summarized and reviewed by Detwiler (4) and by Embree (5).

The success which attended the use of liquid-liquid extraction in the petroleum and other fields suggested its application to fat and oil problems. Thus far the method has been utilized to prepare from vegetable oils fractions more highly unsaturated than the original oils (6, 7); to extract fatty acids from tall oil (8, 9); and to separate known mixtures of pure glycerides, fatty acids, and natural fats (10, 11). Consequently it seemed reasonable to believe that liquid-liquid extraction might be of value in the fractionation of fats and oils with the view of elucidating their component glyceride composition. This communication describes the fractionation of coconut oil by the batch method and by means of a continuous counter-current extractor using 95% ethanol as solvent. During the progress of these studies Oyler (12) investigated the fractionation of olive oil by means of continuous counter-current extraction.

Material and Methods

The coconut oil used in this work was edible oil which had the following physical and chemical constants: neutral equivalent, 661.5; iodine number, 8.5; $n_D^{45°}$, 1.4471.

The batch extractions were carried out in a series of six conical, glass extraction vessels, in which the coconut oil and 95% ethanol were intimately mixed at room temperature by electrically driven spiral glass stirrers. After phase separation by gravity settling the extract layer was withdrawn by siphoning, and fresh solvent added to re-extract the raffinate. In this manner 2.1 kilograms of coconut oil were separated into 43 fractions, including the final raffinate. Preliminary experiments showed that four hours of stirring were necessary to obtain equilibrium between the oil and solvent. Phase separation was allowed to proceed until the extract layer was entirely free of cloudiness. The quantities of solvent used for the first 15 extractions were equal to the estimated weight of raffinate extracted. During the remaining extractions the solvent weight was maintained constant at 1,200 gms., i.e., the weight of the raffinate during the fifteenth extraction.

TABLE I.
Fractionation Data for Methyl Esters of Fatty Acids from Glyceride Fraction No. 3.

Cut	Boiling range °C.	Pressure mm.	Weight gms.	$n_D^{45°}$	Neutral equivalent	Iodine value
1	132-141	40	3.17	1.4068	164.8	
2	141-162	40	5.81	1.4103	172.5	
3	165-167	40	5.33	1.4179	196.8	
4	167-174	40	8.57	1.4208	210.3	
5	179-179	40	11.80	1.4217	215.6	
6	179-182	40	10.36	1.4220	214.8	
7	128-132	2	6.53	1.4228	221.1	
8	132-140	2	7.55	1.4256	233.8	
9	140-142	2	5.99	1.4273	242.4	0.0
10	144-179	2	8.21	1.4331	268.8	25.1
Residue			0.0			
Holdup			1.20			
Cold Trap			1.20			
Loss			0.35			

The continuous counter-current extraction was performed in a slightly modified reflux extraction unit of the type described by Cannon and Fenske (13). The contacting, tower, and disengaging sections were fitted with electric heating coils of chromel ribbon and lagged with asbestos to prevent extreme heat losses. The heat input to the tower and contacting sections was adjusted to maintain their temperature at 40°C. while that of the disengaging section was adjusted so that ethanol distilled over at the rate of three to four milliliters per minute. During the entire period of operation a fine stream of nitrogen gas was injected into the disengaging section. The coconut oil and solvent were allowed to reach equilibrium for 48 hours before a fraction was withdrawn and solvent added. Thus 700 gm. of oil were separated into 32 fractions, including the final raffinate.

Each extract layer was subjected to distillation under reduced pressure to recover the solvent and obtain the extracted glycerides. After measuring the refractive index (at 45°C.) of each glyceride fraction, it was hydrolyzed to separate the free fatty acids. The latter were then converted to the methyl esters and fractionally distilled in a packed column. Typical fractionation data are presented in Table I. The compositions of the fractions obtained by distillation were determined by means of refractive indices, neutral equivalents, and iodine numbers.

[1] Condensed from theses presented by M. H. Menaker and by William R. Fish to the faculty of the Graduate School of Pennsylvania State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy.
[2] This work was supported in part by a grant established by General Mills, Inc.
[3] Present address: Research Laboratories, Swift and Company, Chicago, Illinois.

Results and Discussion

Inasmuch as results obtained by the two methods of extraction were similar in all essential aspects, only those obtained by the batch method are presented.

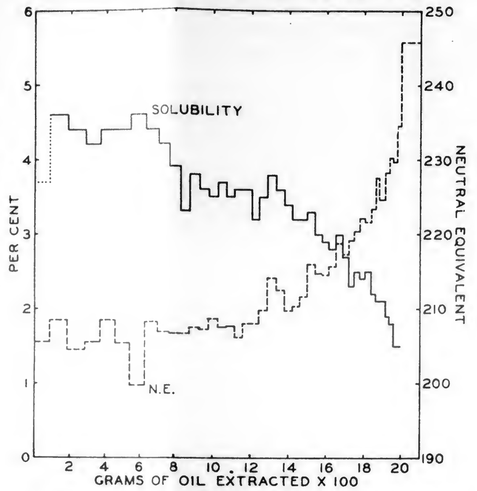

FIG. 1. Variation of solubility of glyceride fractions and neutral equivalents calculated from fatty acid composition of glyceride fractions from coconut oil in relation to weight of material extracted.

The approximate solubilities of the glyceride fractions obtained by the liquid-liquid extraction are presented graphically in Figure 1. The solubility values were calculated by dividing the weight of each glyceride fraction by the weight of solvent used plus the weight of the glyceride fraction. They clearly show that the solubility of the fractions decreased as fractionation progressed. From an initial value of about 4.5% the solubility dropped to about one-third of that at the termination. The low value obtained for the first fraction (dotted line) is due to the fact that much of the ethanol remained dissolved in the fat layer and, after phase separation, did not contribute to the volume of the extract layer. Consequently, the weight of that glyceride fraction was low relative to the quantity of solvent used in the extraction.

Concomitant with the decrease in solubility was an increase in the neutral equivalents [4] (Fig. 1), refractive indices (Fig. 2), and iodine numbers [5] (Fig. 2) of the extraction fractions. All these factors are indicative of a separation of the coconut oil into fractions containing glycerides of progressively larger molecular structure and of increasing unsaturation. The trend of the refractive indices follows very closely that of the iodine numbers, which is in agreement with the fact that in general the introduction of unsaturated linkages into a molecule causes an increase in its index of refraction. It should be noted, however, that the trends of these physical and chemical properties did not commence to change until approximately one-half of the coconut oil had been extracted.

[4] Calculated from fatty acid composition of glyceride fractions.
[5] Calculated from oleic acid content of glyceride fractions.

Inasmuch as the solubility of glycerides containing fatty acids of the acetic acid series is known to decrease with increasing chain length of the acids, it would be expected that the earlier, more soluble glyceride fractions would contain more short-chain acids than the later, less soluble ones; and that the converse would be true with respect to glycerides containing longer-chain acids. In general, this expectation was borne out by the results obtained as will be apparent from an examination of Figure 3. The proportions of the shorter-chain acids (caproic to lauric) in the glyceride fractions decreased as extraction progressed whereas the proportions of longer-chain acids increased.

It is interesting to note from Figure 3 that the glycerides containing oleic acid are found in greater proportions in the later glyceride fractions. This fact would seem to indicate that the solubility of triolein is of the magnitude of the solubility of glycerides containing longer-chain saturated acids. However, no data are available on the solubility of triolein relative to the saturated glycerides; and it cannot be concluded from observations made in the present study whether the solubility of triolein is comparable to that of the longer-chain saturated glycerides or whether triolein compares in solubility with the shorter-chain saturated glycerides but appears in increasing proportions in the later extraction fractions because it is more soluble in the higher glycerides than in the solvent employed.

Although extraction effected a separation of the coconut oil into fractions different in composition from the original oil, all the glyceride fractions contained at least six and in most cases all of the eight fatty acids present in coconut oil. It is possible that this is due, in part at least, to the fact that the component fatty acids of coconut oil may be distributed among the glyceride molecules to a very large degree, in accordance with Hilditch's rule of even distribution. If this were the case, the oil might contain

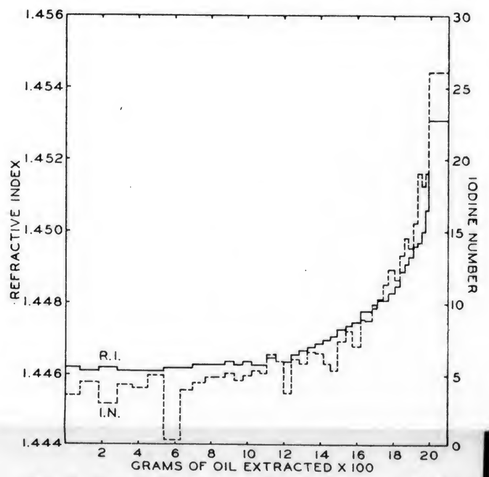

FIG. 2. Variation of refractive indices of glyceride fractions and iodine numbers calculated from oleic acid content of glyceride fractions from coconut oil in relation to weight of material extracted.

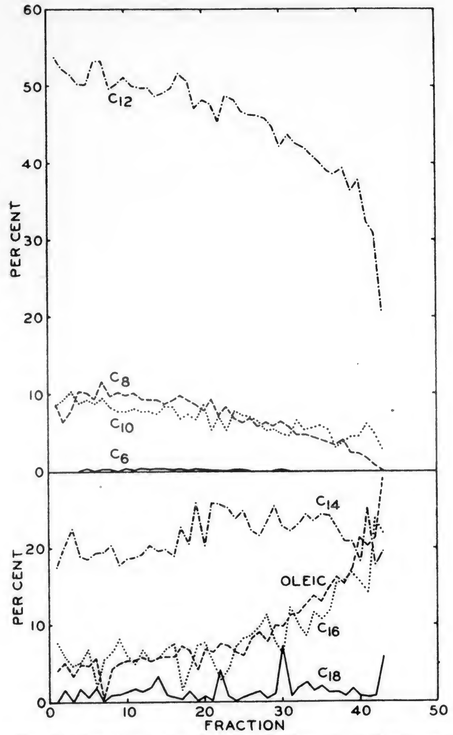

FIG. 3. Distribution of fatty acids throughout fractions obtained by liquid-liquid extraction of coconut oil.

many different glycerides (120 are theoretically possible), the solubilities of which are so similar as to reduce considerably the extent of separation.

When used under the circumstances described in this communication, it is obvious that liquid-liquid extraction is not capable of effecting a separation of fats and oils into fractions simple enough in composition to permit elucidation of their component glyceride composition.

Summary

In order to investigate the value of liquid-liquid extraction as a means of studying the glyceride structure of fats and oils, coconut oil was subjected to liquid-liquid extraction with ethanol, using both the batch method and a continuous counter-current extractor. Both methods yielded essentially similar results.

Extraction effected the separation of coconut oil into fractions different in composition from the original oil, but they were not simple enough in composition to ascertain the glyceride structure of the oil. On the contrary, all the glyceride fractions contained at least six and in most cases all of the eight fatty acids present in coconut oil. In general, proportions of the lower fatty acids (caproic to lauric) in the glyceride fractions decreased as extraction progressed, whereas the proportions of the higher fatty acids increased. These facts were in agreement with changes in solubility, neutral equivalents, refractive indices, and iodine numbers.

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Oxidative Deterioration of Fats in Cereal Products*

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THE PROBLEM of fat deterioration in cereal products presents more complications than a similar deterioration of commercial fats. The possible effect of each component of the cereal product on fat deterioration must be considered. So also must the various procedures involved in the processing of the product, such as mixing, baking, etc., as well as the packaging. Let us consider briefly each of these factors in their relation to fat deterioration.

Shortening Agents. Practically all baked goods as well as prepared mixes contain in their formula one or another of the various commercial fats as shortening agents. It is primarily this added fat which deteriorates and causes the product to spoil. Thus at first glance it would seem logical that the keeping ability of baked products would be related directly to the keeping ability of the shortening used. However, this has not been found to be the case by a number of investigators (Triebold and Bailey [1932-a], Bohn and Olson [1934], and others).

In 1932 Dr. Bailey and myself reported on a study of the keeping ability of 35 commercial cracker samples and the shortenings used in them, secured from as many cracker bakeries scattered over the country. We found that while in general the better keeping crackers were made with shortenings of good keeping qualities, there were several notable exceptions. In certain instances shortenings with poor keeping ability produced crackers with good keeping quality while in other instances the reverse was true.

In an attempt to find an explanation as to why good keeping quality crackers could be produced from a shortening exhibiting poor keeping ability (as indicated by accelerated oxidation studies), lard samples were oxidized to varying degrees of oxygen absorption and used as shortening agents in crackers (Triebold, Webb and Rudy [1933]).

TABLE 1

Comparison of the Length of Induction Period of Experimental Crackers Containing Open Kettle Rendered Lard Oxidized to Different Degrees with the Length of Induction Period of the Lard, and also, the Peroxide Values of the Lards and the Extracted Fat from the Crackers. Length of Induction Periods Determined by Oxidation in a Closed System at 90°C.

	Lard		Crackers	
	Induction period, hours	Peroxide value*	Induction period, hours	Peroxide value*
Control	3.0	1.6	8.0	1.7
Oxidized to beginning of induction period	0.75	3.6	8.0	5.8
Oxidized to 15 cc. O_2 absorption per 100 grams lard	0.0	14.7	8.0	5.0
Oxidized to 50 cc. O_2 absorption per 100 grams lard	0.0	39.5	8.0	3.2

*Ml. 0.002 N $Na_2S_2O_3$ per gram of fat.

The results of this study are tabulated in Table 1. Apparently the pro-oxygens formed by oxidation of the fat are destroyed in the processing of the crackers, and the keeping ability of the fat is restored to its initial fresh condition. Thus, it appears that the inherent keeping ability of the fresh lard, conditioned by its fatty acid composition and structure, is important information to have on a fat. This has been substantiated by Bohn and Olson (1934), who concluded from their work that "in the shortenings themselves we may have stability affected by both factors, antioxidants and pro-oxidants or fatty acid composition. When we make crackers, however, we find that in general stability depends chiefly on the chemical composition of the fatty acids."

Since our usual accelerated oxidation tests determine the keeping quality of a fat as a function of its content of antioxygens and pro-oxygens, as well as its chemical composition, it is obvious why such tests may be in error when relating the keeping quality of a shortening to a baked product in which the shortening is used. Thus there is need for a good test on shortenings to give the anticipated keeping ability of baked goods using those shortenings.

Flour. Twenty years ago the flour salesman blamed the shortening and the shortening salesmen blamed the wheat oil in the flour when the baker's crackers became rancid. This intrigued me at the time and a simple experiment resulted (Table 2, Triebold [1931]).

TABLE 2

Effect of Wheat Oil Present in Cracker Flour on the Keeping Quality of Crackers

Sample	Length of induction period in hours*
Crackers from untreated flour	7.0
Crackers from ether treated (not extracted) flour	7.0
Crackers from extracted flour	3.0
Prime steam lard shortening	4.5

*Crackers and shortening oxidized in a closed system at 90°C.

A flour was extracted with ether while another sample of the flour was treated with ether but not extracted. These two flours, together with the original untreated flour, were baked into crackers using a lard shortening with an induction period of 4.5 hours (subjected to oxidation in a closed system at 90°C.). The crackers from the untreated flour had an induction period of seven hours when subjected to the same oxidizing conditions as the shortening, showing that there were apparently natural antioxidants present in the flour. The ether treated, but not extracted, flour also produced crackers with an induction period of seven hours, showing that the ether treatment did not affect the flour. The ether extracted flour produced crackers with an induction period of 3.0 hours, which was less than the induction period of the original shortening. The presence of natural antioxidants in the ether soluble fraction of cereal products is, of course, well recognized at the present time, and several patented antioxidants of that general source are on the market.

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The possibility of enzymes, particularly oxidase and peroxidase enzymes, accelerating fat oxidation in baked products has been suggested by Carlin and Lannerud (1941). They found that raw potato extract used in a cracker sponge decreased the keeping ability of the resulting crackers. More information on this point would be desirable.

Water. Water has a stabilizing effect on fats (Holm and Greenbank, 1924), on cereals (Fine and Olsen, 1928), and on crackers (Triebold and Bailey, 1932-b). Apparently a certain minimum amount of water is necessary to prevent rapid oxidation of the fat as quantities greater than this amount do not improve the keeping quality. This may mean that the presence of "unbound" or "free" water is necessary to stabilize the product.

Salt. Little information is available relative to the effect of salt on the keeping quality of baked goods. Wilder and Lindow (1937) found that cereal flakes processed without salt were less likely to become rancid than when the normal process involving salt was used. Elder (1941) found that the stability of wheat germ oil was markedly lowered by shaking and cooking with dilute salt brine. Several hypotheses have been advanced to account for this effect, but none have been confirmed. There is need for more information on this subject.

Sugar. Bohn and Olson (1934) state that sugar is a powerful antioxidant. However, the quantity of sugar required to show strong antioxidant properties is so large that it could never be added solely for that purpose. The stability imparted by the sugar is incidental to the use of sugar as a sweetening agent in certain products. This is no doubt one of the reasons why rancidity in cookies is not as prevalent as in crackers.

McKinney and Bailey (1941) also found sugar to have a strong antioxidant effect when added to a biscuit formula in the amount of one-fifth of the weight of the flour used in the formula. They found sugar to exert a protective factor of 5-6 when oleo oil, hydrogenated lard, or hydrogenated vegetable oil was used as a shortening agent in the formula. Sugar only exerted a mild antioxygenic effect (protective factor of 1.5-1.6) when used with prime steam lard in the formula.

Antioxidants. The addition of antioxidants to fats has been found in a number of cases to improve tremendously the keeping qualities of the fats. However, the carry-over of this effect into baked products has not been too satisfactory.

Bohn and Olson (1934) experimented with two antioxidants used for stabilizing lard and found that while both were effective in the lard itself, the one was completely lost when the lard was used in crackers. The other was somewhat effective in the crackers produced, which however still lacked the keeping qualities of crackers produced with good hydrogenated shortenings.

Oat flour and oat flour extracts were found to have but a slight favorable effect on the keeping qualities of crackers (Triebold, 1938). The oat flour and extracts were added to the cracker sponge, in doughing up the sponge, or sprayed or dusted upon the baked crackers. A protective factor from 0 to 2, which for all practical purposes is negligible, was exerted by the various treatments.

Lundberg, Halvorson, and Burr (1944) found that NDGA (nordihydroguaiaretic acid) when added to a lard used in making pie crusts and soda crackers exerted some stabilizing effect on the resulting product (protective factor of approximately 2 in crackers and 10 in pie crusts). The lesser effectiveness of the NDGA in crackers was thought to be due to the alkalinity imparted by the baking soda since alkaline solutions of NDGA oxidize rapidly when exposed to air.

Higgins and Black (1944) studied the effect of several antioxidants added to lard used in the preparation of crackers. They found gum guaiac to be an effective antioxidant for lard with the stability carrying over into the baked product (protective factors of 2.5-7, depending upon concentration). Propylgallate exerted a stabilizing effect on the lard but practically none on the resulting crackers. The same was also true for the tocopherols and for a wheat germ oil derivative (an ethylene dichloride extract of wheat germ oil combined with citric acid).

Mixing, Fermentation and Baking. These manipulative procedures involved in the manufacture of baked goods likely play a role in the keeping quality of the resulting product. This has been referred to previously in the destruction of pro-oxidants and antioxidants present in a fat when baked into crackers. Mixing spreads the fat over a greater area and also in the presence of salt may cause the solution of certain fat components into the aqueous phase, thereby facilitating their oxidation later. Fermentation produces sugars and organic acids, and these may have an effect upon the stability of the shortening.

The temperature and length of baking time might be anticipated to have an effect on the stability of the fat in baked products. Apparently as long as there is sufficient moisture present so that the product does not scorch, the effect is not great. However, crackers with scorched spots or crackers that have been crisped by successive reheatings show a marked decrease in keeping quality.

Packaging. The possibility of the absorption of fat from a baked product by the package must not be overlooked. The lining of cracker packages with grease-proof paper has helped greatly. However, some baked products are packaged in cardboard boxes. In such packages the fat may be absorbed rapidly by the cardboard, thereby spread over a large surface, and consequently undergoes rapid oxidation. This emphasizes the need for proper packaging to insure a good keeping quality product.

Several patents have been taken out on the impregnation of cardboard packages and wrappers with antioxidants and these are used to some extent. In certain instances these have proved helpful in retarding spoilage of the products contained, while in other instances they have been ineffective.

The use of colored glass to cut out the blue and ultraviolet rays of light was suggested by Burr (1907) as a means of protecting dairy products from oxidative deterioration. This has led to the development and effective use of colored cellophanes for packaging food products that will be exposed to light when merchandized.

Summary. In summarizing, it would appear that effects on the stability of cereal products to oxidative deterioration by formula components as well as methods of processing, are not understood to the degree that they should be and that there is a great need for studies along these lines.

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[Contribution from the Wyeth Institute of Applied Biochemistry, and the Department of Agricultural and Biological Chemistry, The Pennsylvania State College]

The Synthesis of Some Organic Compounds of Gold

By Charles W. Denko[1] and Arthur K. Anderson

Several organic gold compounds were desired by us for a series of experiments on the physiological effects of gold compounds.[2] Two types of compounds, gold alkylxanthates ROCSSAu and aliphatic gold thio acids RCH(SAu)COOH, were synthesized by procedures based on the methods outlined by Zeise, Scala and Biilman.[3] The gold alkylxanthates were prepared thus

$$ROH + CS_2 + KOH \longrightarrow ROCSSK \xrightarrow{AuCl_3} ROCSSAu + ROCSS{\cdot}SSCOR$$

The aliphatic gold thio acids were prepared as indicated

$$RCH(X)COOH \xrightarrow{Na_2CO_3} RCH(X)COONa \xrightarrow{C_2H_5OCSSK}$$

$$RCHCOONa \xrightarrow{HCl} RCHCOOH \xrightarrow{NH_4OH}$$
$$\quad | \qquad\qquad\quad |$$
$$SSCOC_2H_5 \qquad SSCOC_2H_5$$

$$RCH(SH)COONH_4 \xrightarrow{HCl} RCH(SH)COOH \xrightarrow{AuCl_3}$$
$$+$$
$$NH_2S{-}COC_2H_5$$

$$RCH(SAu)COOH \xrightarrow[MCl]{MOH} RCH(SAu)COOM$$

Table I lists the gold compounds prepared, together with their formulas, melting points and gold content, the gold content being an index of purity.

Experimental

Gold Alkylxanthates.—These compounds were prepared according to methods of Zeise[3a] and Scala.[3b] Potassium alkylxanthates were prepared by dissolving potassium hydroxide in the appropriate alcohol and adding carbon disulfide. All have a characteristic odor and pale yellow color, decompose on heating, and are very soluble in water.

In preparing the gold alkylxanthate a 10% solution of auric chloride (as $HCl{\cdot}AuCl_3{\cdot}4H_2O$) was added dropwise to a 25% solution of potassium ethylxanthate with constant stirring. A three-fold excess by weight of ROCSSK was used. If an excess of auric chloride is used or if the C_2H_5OCSSK is added to auric chloride there is more difficulty in obtaining a pure product. The oily gold alkylxanthate was filtered off, washed several times with water and ether leaving a yellow powder with a slightly fruity odor. The product was kept in amber bottles to prevent fading due to light. The compounds are insoluble in water and the common organic solvents. Yields are practically quantitative (97–98% of theoretical).

Aliphatic Gold Thio Acids.—The appropriate α-halogen acids were used to prepare α-thiol acids using the method of Biilman[3c] as outlined in the previous series of reactions. Forty eight grams of α-bromobutyric acid yielded 16 g. of α-thiobutyric acid, b. p. 120–123° at 18 mm. (53% theoretical yield). The acid had a somewhat unpleasant odor and solidified after standing in the refrigerator a few hours.

To a 10% solution of α-thiobutyric acid was added dropwise with constant stirring, a 10% solution of auric chloride, forming a light yellow flocculent precipitate, α-gold thiobutyric acid. If an excess of auric chloride is added the precipitate becomes dark brown. The yellow precipitate was washed several times with water and dried. Yields are practically quantitative. α-Gold thiobutyric acid forms a soluble sodium salt and an insoluble calcium salt. The acid is soluble in absolute ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *i*-butyl and 95% ethyl alcohol; insoluble in ether, carbon tetrachloride, petroleum ether and carbon disulfide. At 100°, 0.055 g. dissolved in 100 ml. of water.

By a similar method starting with monochloroacetic acid, α-thioglycolic acid was prepared with some of its salts. The potassium, ammonium and sodium salts of gold thioglycolic acid are soluble in water whereas the calcium and strontium salts are insoluble.

Table I

Properties of Organic Gold Compounds

	Compound	Formula	M. p., °C.	Gold % Found	Gold % Calcd.
	Gold alkylxanthates				
1	Methyl	$CH_3OCSSAu$	160 *ca.*	64.45	64.81
2	Ethyl	$C_2H_5OCSSAu$	165–168	61.64	61.94
3	*n*-Propyl	$C_3H_7OCSSAu$	165–167	59.16	59.29
4	*i*-Propyl	$C_3H_7OCSSAu$	163–165	59.42	59.29
5	*n*-Butyl	$C_4H_9OCSSAu$	162–164	57.78	56.92
6	*i*-Butyl	$C_4H_9OCSSAu$	162 *ca.*	56.89	56.92
	Gold thio acids				
1	Strontium gold thioglycolate	$(CH_2SAuCOO)_2Sr$	240–280	60.61	59.53
2	α-Gold thiobutyric acid	$CH_3CH_2CH(SAu)COOH$	187–197	61.40	62.24

Summary

1. The following organic gold compounds have been prepared: gold alkylxanthates, methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, *i*-butyl; strontium gold thioglycolate and α-gold thiobutyric acid.

2. Some reactions and physical properties of these gold compounds are noted.

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(2) Denko and Anderson, *J. Lab. Clin. Med.*, **29**, 1168 (1944).

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